IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

36761

MGIPC—S4—[illegible]93—22-8-45—5,000

CHEMICAL REVIEWS

EDITORIAL BOARD
W. Albert Noyes, Jr., Editor
Louise Kelley, Assistant Editor
J. G. Kirkwood L. A. Maynard
E. H. Volwiler I. M. Kolthoff
H. F. Johnstone C. S. Hamilton

VOLUME 38

PUBLISHED BI-MONTHLY FOR
The American Chemical Society
BY
The Williams & Wilkins Company
Baltimore, U. S. A.
1946

36761

CONTENTS

NUMBER 1, FEBRUARY, 1946

The Antoine Equation for Vapor-pressure Data. GEORGE WM. THOMSON.. 1
Magnetism and Catalysis. P. W. SELWOOD.......................... 41
The Reactions of Unsaturated Ketones and Derivatives with Amino Compounds. Amino Ketones. NORMAN H. CROMWELL.................. 83
The Ullmann Synthesis of Biaryls. PAUL E. FANTA..................... 139

NUMBER 2, APRIL, 1946

Oxidation of Lubricating Oils. H. H. ZUIDEMA.......................... 197
The Synthesis of Aromatic Aldehydes. LLOYD N. FERGUSON............. 227
Metabolite Antagonists. RICHARD O. ROBLIN, JR....................... 255

NUMBER 3, JUNE, 1946

Ferritin: Its Properties and Significance for Iron Metabolism. S. GRANICK. 379
The Chemistry of the Diaryl Ethers. HERBERT E. UNGNADE............. 405
Aromatic Cyclodehydration. CHARLES K. BRADSHER.................... 447
The Isolation and Synthesis of the Naturally Occurring α-Amino Acids. RICHARD J. BLOCK.. 501

THE ANTOINE EQUATION FOR VAPOR-PRESSURE DATA

GEORGE WM. THOMSON

Ethyl Corporation, Detroit, Michigan

Received May 23, 1945

The vapor pressure–temperature relation may be represented in a variety of ways, none of which is completely satisfactory. Of the simpler relations involving two or three empirical constants none is able to represent the temperature relation faithfully over the entire liquid range. However, many are useful for interpolations and extrapolations, particularly below or above a reduced temperature of 0.75. The commonly used equation

$$\log P = A - B/T$$

has not been found reliable, except for very short temperature ranges or for low-boiling substances. On the other hand, the Antoine equation

$$\log P = A - B/(t + C)$$

has been found to be entirely adequate for data of all but the very highest precision, from the triple point to a reduced temperature of 0.75. This equation introduces a needed correction to the commonly used equation (which may be written in the form $\log P = A - B/(t + 273.16)$), since the best value of C is not necessarily equal to 273.16 and in fact is usually much lower.

The Henglein equation:

$$\log P = A - B/T^n$$

is similarly useful over the same range but is more difficult to use. The Antoine equation is thus recommended for general use and simple means are described for obtaining the empirical constants, including approximate rules for estimating the value of C.

Of the equations which are designed to fit over the entire liquid range, those of Cox, Biot, and Gamson and Watson are considered most satisfactory. For practical purposes two Antoine equations, one up to a reduced temperature of 0.75, the other from that point to the critical point and both tangent at their intersection point, are much more convenient to use and are fully as accurate.

A method is presented for calculating the heat of vaporization from vapor-pressure data, using the Antoine equation. This method includes a suitable correlation for estimating the effect of the volume of the liquid compared with the vapor and the deviation of the vapor from the perfect gas laws.

A nomograph is given which is based on the use of $C = 230$, a good average value for organic compounds which are liquid at room temperature. This chart may be used for rapid interpolations and also for the estimation of vapor pressures from meager information.

I. INTRODUCTION

Many different formulas and representations have been presented for the vapor pressure–temperature relationship for pure liquids and their mixtures. This is due not only to the importance of this physical property itself but also to its relation to other thermodynamic properties, such as the latent heat of vaporization. For use in thermodynamic calculations an interpolation formula of some kind is well-nigh essential, particularly since the experimental data are usually fragmentary and located at inconvenient temperature and pressure intervals. The purpose of this paper is to review these representations of vapor-pressure data, with special emphasis on the Antoine equation.

II. FORMULAS IN USE IN THE PAST

The very earliest vapor-pressure formula was given by Dalton (17), who observed that the pressures increased in geometric progression while the temperatures increased in arithmetic progression, that is:

$$\log P = a + bT \tag{1}$$

This relation was quickly disproved when better measurements became available, but the gentle curvature of vapor-pressure data plotted on semi-log paper demonstrates the approximate validity of the rule.

III. RELATIONS BASED ON CLAUSIUS–CLAPEYRON EQUATION

Many of the formulas in use have a semi-theoretical background, often being based on the Clausius–Clapeyron equation:

$$\frac{dP}{dt} = \frac{\Delta H}{T\Delta V} = \frac{\Delta H}{T(V_g - V_l)} \tag{2}$$

where P = vapor pressure of liquid,
t = temperature,
T = absolute temperature,
ΔH = heat of vaporization,
ΔV = change in volume on vaporization,
V_g = molecular volume of gaseous phase, and
V_l = molecular volume of liquid phase.

The most familiar vapor-pressure formula is probably the relation:

$$\log P = A - \frac{B}{T} \tag{3}$$

This "$1/T$ form" has a semi-theoretical background, being the integrated form of the Clausius–Clapeyron equation with the assumptions that: (*1*) the volume of the liquid, V_l, is negligible compared with the volume of the vapor, V_g; (*2*) the heat of vaporization, ΔH, is constant over the temperature range involved; and (*3*) the vapor is a perfect gas. Obviously these three assumptions are only crude approximations, unless the temperature range involved is small indeed, but the combination of all three leads to a much better approximation because some of the errors cancel. This may be more clearly seen by writing the Clausius–Clapeyron equation in the following form:

$$\frac{d \ln P}{d(1/T)} = -\frac{1}{R}\cdot\frac{\Delta H}{\Delta z} \tag{4}$$

where

$$\Delta z = \Delta z_g - \Delta z_l$$

$$\Delta z_g = PV_g/RT$$

$$\Delta z_l = PV_l/RT$$

The Δz term provides a convenient double correction for both the non-ideality of the vapor and the effect of including the liquid volume in the ΔV term. The ratio $\Delta H/\Delta z$ does not vary very much with temperature, so that for approximate vapor-pressure data it appears to be constant and the $1/T$ form seems to apply. However, application to more accurate data shows a definite variation of $\Delta H/\Delta z$ with temperature, so that, in general, the $1/T$ form does not give a true picture of the vapor pressure–temperature relation. Deviations exceed reasonable experimental error and their systematic trends indicate that extrapolation is unwise even over short ranges.

The assumption that the heat of vaporization is constant was early seen to be one of the chief contributing factors in the inability of the $1/T$ formula to represent the data within the experimental error. To avoid this difficulty the heat of vaporization was taken to be one of several various functions of the temperature, giving corresponding vapor-pressure equations. For example: if the volume of the liquid is neglected compared with the vapor, the vapor is considered to be a perfect gas, and the heat of vaporization is represented by a polynomial such as:

$$\Delta H = \Delta H_0 + bT + dT^2 + eT^3 + \cdots \tag{5}$$

then the vapor-pressure formula becomes

$$\log P = \frac{1}{2.3R}\left\{a - \frac{\Delta H_0}{T} + 2.3b \log T + dT + \frac{e}{2} T^2 + \cdots\right\} \tag{6}$$

where $2.3 = \ln 10$ and $2.3R = 4.57566$ for ΔH in calories per mole. One of the simpler forms with $d, e, \cdots = 0$ is generally attributed to Kirchhoff (30), but Rankine (42) used it nine years earlier.

The assumptions involved in equation 5 may be replaced by the single relation:

$$\Delta H/\Delta z = \Delta H_0 + bT + dT^2 + eT^3 + \cdots. \tag{7}$$

which may be valid up to the critical point, although the individual assumptions are not.

The constants of equation 7 are directly related to the difference in specific heats of the two saturated phases, ΔC_s, but the rigorous relation (19) is complex:

$$\Delta C_s = \Delta C_p - T\frac{dP}{dt}\left\{\left(\frac{\partial V_g}{\partial T}\right)_P - \left(\frac{\partial V_l}{\partial T}\right)_P\right\} \tag{8}$$

also

$$\Delta C_s = \frac{d\Delta H}{dT} - \frac{\Delta H}{T} \tag{9}$$

where ΔC_p = difference in isobaric specific heats of liquid and vapor, and
ΔC_s = difference in specific heats of the saturated liquid and vapor phases.

The rigorous application of these equations is seldom possible because of incomplete P–V–T data. Below the normal boiling point the following approximation is frequently sufficiently accurate to provide suitable vapor-pressure relations:

$$\Delta C_p = \frac{\partial}{\partial T}\left(\frac{\Delta H}{\Delta z}\right) \tag{10}$$

If the isobaric specific heats of liquid and vapor are available, then the constants of the vapor-pressure equation (equation 6) are easily determined.

In his classical work, Nernst (37) derived a similar formula on the following assumptions:

$$\Delta z = \frac{P\Delta V}{RT} = 1 - \frac{P}{P_c} \tag{11}$$

$$\Delta H = (\Delta H_0 + \beta T - \epsilon T^2)\left(1 - \frac{P}{P_c}\right) \tag{12}$$

where P_c = critical pressure. Substitution into the Clausius–Clapeyron equation and integration gives:

$$\log P = \frac{\Delta H_0}{2.3RT} + \frac{\beta}{2}\log T - \frac{\epsilon T}{2.3R} + C \tag{13}$$

In equation 13, ΔH_0, β, ϵ, and C are empirical constants. Nernst found that β is often close to 3.5 and that C appeared to be independent of the state of the compound. He therefore called C a "chemical constant". There was considerable interest in these constants which stimulated interest in vapor-pressure work. In particular, the constants were used to calculate reaction equilibria until they were supplanted by more accurate thermodynamic methods.

This equation has been very widely used, since the addition of the extra two terms to the $1/T$ form removes many of its disadvantages, although the assumptions are not valid and the relation is purely empirical. Equation 11 satisfies the boundary conditions at zero pressure and the critical point but gives values of Δz which are consistently low. The calculated values are only about 50 per cent of the experimental values (34) above $P_R = 0.7$. Fortunately this error is not reflected in the final Nernst vapor-pressure equation, since the quantity $(1 - P/P_c)$ cancels out.

At low pressures, where equation 10 can be applied, the Nernst equation gives

$$\Delta C_p = \beta - 2\epsilon T\text{, or} \tag{14}$$

$$= 3.5 - 2\epsilon T \tag{15}$$

Since it is known that ΔC_p is usually negative and, for inorganic salts, about − 3.5 per atom in the molecule[1], ϵ will usually be larger than 0.001. Another approximate check on the Nernst equation is obtained from the value of the "chemical constant", C, which, for all "normal" liquids, should be about 6.0 for P in millimeters, although low-boiling substances are much lower (hydrogen = 4.5; nitrogen = 5.4) and associated liquids are somewhat higher (water = 6.5).

---

[1] This is the value usually used by K. K. Kelley for estimating ΔC_p in his vapor-pressure compilations (29).

IV. EMPIRICAL EQUATIONS

Another school of thought broke away from the Clausius–Clapeyron equation tradition and proposed frankly empirical vapor-pressure equations, some of which are shown below. An excellent review of many of those proposed prior to 1910 is presented in Chwolson's *Traité de physique* (13). No simple vapor-pressure equation has yet been devised which fits the data from the triple point to the critical point and which is also in agreement with thermodynamic data on the saturated phases.

Bose (8) proposed the equation

$$\log P = a - \frac{b}{T} + \frac{d}{T^2} + \frac{e}{T^3} \tag{16}$$

which he claimed to be better at high temperatures than the Nernst equation.

A different attack on the problem was that of Biot (7), whose exponential equation

$$\log P = a + b\alpha^t + c\beta^t \tag{17}$$

was used by Regnault for much of his early work and more recently by Sidney Young (57) to fit vapor-pressure data for a variety of compounds up to the critical point. By expansion this equation may be shown to be equivalent to a convergent infinite series of the type:

$$\log P = A + BT + DT^2 + \cdots \tag{18}$$

Many other similar exponential forms are encountered.

van der Waals proposed the relation

$$\log \frac{P_c}{P} = a\left\{\frac{T_c}{T} - 1\right\} \tag{19}$$

where a was supposed to be a universal constant, but this was soon shown to be inaccurate, since a depends on both temperature and substance (38).

Carbonelli suggested the modification

$$\log \frac{P_c}{P} = \left\{\frac{T_c}{T} - 1\right\} \log\left(b\,\frac{T_c}{T}\right) \tag{20}$$

where $\log b$ lies between 2 and 4. This equation does not give a reasonable representation of the data, since b is a parabolic-type function of temperature, instead of a constant, as claimed.

The variation of these "constants" from substance to substance in equations involving "reduced" properties ($P/P_c = P_R$ = reduced pressure, for example) is in line with the well-demonstrated fact that there is no general equation of state possible for compounds along the saturation line.

Several workers have used the form

$$\log P(\text{atm.}) = A\left(1 - \frac{T_B}{T}\right) \tag{21}$$

where A is some function of the temperature and substance. One of the most successful studies using this method was made by Cox (16), who showed that log A was a parabolic function of temperature over the entire liquid range for all the compounds tested, including hydrocarbons, hydrogen, oxygen, nitrogen, carbon dioxide, water, ammonia, and several alcohols and ethers. The fundamental relationship can be written

$$\log A = \log A_c + E(1 - T_R)(F - T_R) \tag{22}$$

where A_c = value of A at critical point,
T_R = reduced temperature,
E = empirical constant, a linear function of boiling point for the paraffins, and
F = empirical constant, equal to 0.85 for hydrocarbons with more than two carbon atoms.

This method of calculation provides an excellent check on the accuracy of vapor-pressure data, since a plot of A (or log A) against temperature should be smooth. In practice the plot is found to be very sensitive near the boiling point, the critical point, and near $T_R = F$, but this sensitivity is of little importance in determining either E or F. This equation is important, both because it provides a good fit over the entire liquid range with but few empirical constants and also because the fundamental parabolic relation which appears to be established provides an excellent check on other suggested equations which profess to fit over the the entire liquid range.

The recent work of Gamson and Watson (21) presents a similar ambitious attempt to provide a vapor-pressure equation which is valid over the entire liquid range. They modify the $1/T$ form by adding an exponential term, as follows:

$$\log P = A - \frac{B}{T} - e^{-20(T_R - b)^2} \tag{23}$$

Methods are presented for estimating the values of b and the critical constants for fifteen homologous series of organic compounds. Since the exponential term becomes negligible at the higher temperatures, the equation approaches the $1/T$ form near the critical point. However, the work of Cox (16) described above and similar studies by the author have shown that in general the vapor pressure–temperature relation does not follow a $1/T$ form equation even at the high range. If the $1/T$ form were applicable then:

$$\frac{T_c \cdot T(y_c - y)}{T_c - T} = \text{a constant, } B \text{ in equation 3} \tag{24}$$

However, plots of this quantity against temperature, for various compounds, show pronounced minima near $T_R = 0.75$ instead of leveling off near the critical point as predicted by the Gamson and Watson relation, which may be written:

$$\begin{aligned} \frac{T_c \cdot T(y_c - y)}{T_c - T} &= B + \frac{T \cdot T_c}{T_c - T} e^{-20(T_R - b)^2} \\ &= B + \frac{T_c \cdot T_R}{1 - T_R} e^{-20(T_R - b)^2} \qquad (25) \end{aligned}$$

The only minimum for this function occurs at the critical point, so that, like most other empirical vapor-pressure equations, the Gamson and Watson relation cannot fit exactly over the entire liquid range, although the approximation is very good indeed. When applied to accurate experimental data the equation provides a remarkably good fit up to about $T_R = 0.80$ and only a small systematic error, of the order of 1 to 2 per cent, from that temperature to the critical point.

It is unfortunate that this useful equation is difficult to handle. The exponential term is not readily calculated and yet it constitutes a large part of the value of log P at the lower temperatures (e.g., 20 per cent of log P for n-heptane at 0°C.). Also, the temperature cannot be obtained from the pressure without a trial solution. The high accuracy of both this equation and the Antoine equation (see below) suggests that an excellent procedure for calculating vapor pressures over the entire liquid range would be to fit a Gamson and Watson equation to the data and calculate from it five or six points, as follows: one or two below the boiling point, at the boiling point, and at $T_R = 0.8$, 0.9, and 1.0. Two Antoine equations could then be fitted, one up to $T_R = 0.8$, the other from $T_R = 0.8$ to the critical point. This procedure combines the long-range accuracy of the Gamson and Watson equation with the precision and ease of calculation of the Antoine equation.

The use of polynomials in t (or P) to give P (or t), such as have been employed by the Bureau of Standards for their accurate measurements on water, heptane, and isoöctane, is convenient in that no logarithm tables are required. For example, their equations for isoöctane (51)

$$P = 760 + 21.500(t - b) + 0.2348(t - b)^2 + 0.00126(t - b)^3 \qquad (26)$$

where $b = 99.234$, the normal boiling point, and

$$\begin{aligned} t = 99.234 &+ 0.046511(P - 760) \\ &- 0.0_4 2374(P - 760)^2 + 0.0_7 182(P - 760)^3 \qquad (27) \end{aligned}$$

give a very close fit to their data over the range from 660 to 860 mm. Equation 27 gives t with a maximum deviation of only 0.003°C. However, when the work was repeated over a wider range (49) the Antoine equation (see below)

$$\log P = 6.820137 - \frac{1262.707}{t + 221.307} \qquad (28)$$

was found to give a much better representation from 100 to 1500 mm.

When a very accurate formula is desired, as that for a primary ebulliometric standard, very elaborate means may be employed. For example, Osborne and Meyers (40) found it necessary to employ the following equation for the vapor pressure of water over the entire liquid range:

$$\log P = A - \frac{B}{T} + \frac{Cx}{T}\,(10^{Dx^2} - 1) - E(10^{Fy^{5/4}}) \tag{29}$$

where $T = t + 273.16$,
$x = T^2 - K$, and
$y = t_c - t$, where $t_c = 374.11°\text{C.}$, critical temperature, and the parameters have the following values:

$A = 5.4266514$
$B = 2005.1$
$C = 1.3869 \times 10^{-4}$
$D = 1.1965 \times 10^{-11}$
$E = 0.0044$
$F = -0.0057148$
$K = 293{,}700$

The authors stated that "the resulting formula here presented makes no claim to rationality or apparent simplicity, as these qualities have as yet eluded the inspiration of the authors and their colleagues."

In almost all the equations mentioned the temperature enters the formula as T, the absolute temperature. Various investigators broke from tradition by modifying the terms in T. Ashworth (4) plotted $\log P$ *vs.* $1/(\sqrt{T^2 + 108{,}000} - 307.6)$ for normal paraffins (C_5H_{12} to $C_{32}H_{66}$) and obtained straight lines with a common intersection point at 1249°C. and 3.63×10^5 mm. Henglein (24) used an exponent on the T in the $1/T$ form giving:

$$\log P = A - B/T^n \tag{30}$$

The proper choice of n gives a fairly good fit over a large temperature range. Henglein considered that A was a universal constant, equal to 7.5030 for P in millimeters. On the other hand, in developing a vapor-pressure slide rule, Miles (35) considered that, although n varied from 1.05 to 1.56, an average value of 1.3 was suitable for all compounds. Actually the equation, with a proper choice of all three constants, gives a very good fit to the experimental data at the lower temperatures, but diverges appreciably above reduced temperatures of 0.75 to 0.80. Thus, for the S. Young (57) data on n-heptane the equation

$$\log P = 6.33546 - 11877.85/T^{1.376} \tag{31}$$

fits to about ±0.2 per cent in pressure, or to about ±0.1°, up to 160°C. ($T_R = 0.80$). Above this temperature the equation has an increasingly larger systematic error until at the critical point (266.85°C.; 20,430 mm.) the calculated values are 7.8° high and 9 per cent low, respectively. The Antoine equation (see below) through the same data gives a comparable fit over the same range and a somewhat lower systematic error, which reaches a maximum of 4.2° in temperature and 5 per cent in pressure at the critical point.

The Henglein equation is believed to be reliable for data either below or above $T_R = 0.75$ to 0.80, but not for data both above and below this temperature, unless the range is very short. The equation is cumbersome to handle but gives direct solutions for both temperature and pressure. Unfortunately the constant A is not universal, as Henglein claimed, so that all three constants must be determined.

V. THE ANTOINE EQUATION

Various disadvantages are associated with the use of many of the formulas described above. The $1/T$ form suffers from the fact that its fundamental assumptions do not hold. The problem of the proper value of the ice point influences the value of the formula for precise work. Some of the values used are 273, 273.09, 273.1, 273.2, 273.13, 273.16, and 273.167. Unfortunately, many authors do not even say what their ice point is. When log P is plotted against $1/T$, the data are almost always bowed, indicating that extrapolation is particularly hazardous.

The more complicated formulas represent the data much better, but there are some difficulties connected with their use. The empirical constants are usually tedious to determine. In many cases, it is difficult to find t as a function of P, as with the equations of Nernst, Bose, Biot, Cox, Gamson and Watson, and the complicated water equations of the Bureau of Standards, where a trial-and-error or graphical solution must be resorted to. The number of constants used are often more than are justified by the precision of the data.

The Bureau of Standards polynomials for t and P are excellent for representing the data over limited ranges but are somewhat tedious to calculate.

The much simpler Antoine equation, originally applied to water (1), simply substitutes $(t + C)$ for T in the simple $1/T$ form giving:

$$\log P = A - \frac{B}{t + C} \tag{32}$$

In a later publication (2) Antoine applied the equation to over twenty compounds and mixtures. A similar extensive study on one hundred fourteen liquids and twenty-seven solids was made by Schmidt (45), who was apparently ignorant of Antoine's work. When applied by Calingaert and Davis (10) to several classes of compounds (metallic elements, hydrocarbons, alcohols, acids, etc.), using $C = 230$, good straight lines were obtained on the plot of log P *vs.* $1/(t + C)$ with an intersection point for each group studied. The hydrocarbon point is at 1240°C. and 1.3×10^6 mm., not very different from that found by Ashworth, whose method of plotting is approximately equivalent to the use of $C = 150$. Since for most classes of compounds the value of C decreases with increasing boiling point, the charts with intersecting straight lines[2] are only approximately correct. However, this chart error is frequently less than the other uncertainties of the data and the choice of $C = 230$ is fortunate in that it is a good middle value. Many later workers have used the Antoine equation for long-range data of moderate precision and for shorter-range data of high precision, with considerable success. For example, the Bureau of Standards equation for isoöctane (equation 28) reproduces the observed pressures with an average deviation of 0.05 mm. from 100 to 1500 mm. pressure.

---

[2] These charts are usually known as Cox charts. Cox (15) plotted log P against the logarithm of the vapor pressure of water at the same temperature.

The Antoine equation possesses various advantages when compared with the other types of vapor-pressure equations in the literature. It fits every case tried better than the $1/T$ form, excepting low-boiling compounds and monatomic elements, where the value of C is close to 273. Extrapolation is safer than with the $1/T$ type, because the use of a constant other than the ice point, 273, reduces the systematic deviations which cause bowing of the straight line expected when $\log P$ is plotted against $1/(t + C)$. The value of t is readily obtained from P. The constants are easily determined. In many cases which have been studied C lies between 220 and 240, and $C = 230$ may be used for many compounds. This reduces the Antoine equation to a readily handled two-constant equation, linear in $\log P$ and $1/(t + 230)$.

In many examples of data of moderate precision (0.1–2 per cent in P), the Antoine equation reproduces the values closer than the equations suggested by the workers, which are usually more complicated. Several comparisons are shown in a later section.

A striking example in the field of high-precision measurements is the Antoine equation for water suggested by Swietoslawski and Smith (52),

$$\log P(\text{atm.}) = 5.053988 - \frac{1647.6}{t + 226} \qquad (33)$$

which reproduces the values from 80° to 100°C. with as high precision as the more complex formula of Osborne and Meyers (equation 29).

An useful scheme for estimating vapor pressures is the application of the Calingaert and Davis work described above. Since groups of similar compounds have often a common intersection point when $\log P$ is plotted against $1/(t + 230)$, the intersection point and a boiling point can be used to estimate the entire vapor pressure–temperature curve. This method was applied in this laboratory to the vapor pressures of the R_4Pb compounds (9) where there are good long-range data on both $(C_2H_5)_4Pb$ and $(CH_3)_4Pb$, but only scanty data on the other three lead alkyls, $(CH_3)_3C_2H_5Pb$, $(CH_3)_2(C_2H_5)_2Pb$, and $CH_3(C_2H_5)_3Pb$.

The Antoine equation can also be used to calculate the heat of vaporization quite precisely, as will be shown below.

Although the Antoine equation is certainly a most satisfactory three-constant vapor-pressure equation for interpolation purposes, it can not be extrapolated above a reduced temperature (T_R) of about 0.85 without serious error. However, two Antoine equations, one up to $T_R = 0.8$ or 0.85 and the other from $T_R = 0.8$ to $T_R = 1.0$, preferably through the critical point and contacting the low-range equation at $T_R = 0.8$, are usually perfectly adequate to cover the entire liquid range.

As has been described above, Cox showed that for all the compounds tested, his quantity, $A_{\text{Cox}} = T \log P$ (atm.)$/(T - T_B)$, gradually decreases with increasing temperature with a minimum at $T_R = (F + 1)/2$ (about 0.925 for most hydrocarbons), then increases until at the critical point the value of A equals the values at $T_R = F$ (about 0.85). It is easily shown that if the vapor pressures follow an Antoine equation, then for P in atmospheres:

$$A_{\text{Cox}} = A_{\text{Antoine}}\ (T/(t + C)) \tag{34}$$

Since this equation can never have a minimum, it follows that the Antoine equation cannot possibly fit from low temperatures right up to the critical.

TABLE 1

Comparison of Antoine and Cox equations, using S. Young's data (58) for n-pentane

P	LOG *P*	BOILING POINT				
		Young	Antoine equation		Cox equation	
		t	*t*	*Difference*	*t*	Difference
mm.		*°C.*	*°C.*	*°C.*	*°C.*	*°C.*
11	1.0414	−49.4	−49.2	+0.2	−49.2	+0.2
15	1.1761	−44.9	−44.8	+0.1	−44.8	+0.1
30	1.4771	−34.2	−34.2	0.0	−34.2	0.0
50	1.6990	−25.6	−25.6	0.0	−25.6	0.0
100	2.0	−12.7	−12.8	−0.1	−12.7	0.0
199.5	2.3	+1.7	+1.8	+0.1	+1.8	+0.1
398.1	2.6	18.3	18.3	0.0	18.3	0.0
760	2.880	36.1	36.1	0.0	36.1	0.0
1000	3.0	44.4	44.4	0.0	44.4	0.0
1995	3.3	67.9	67.8	−0.1	67.8	−0.1
3981	3.6	95.6	95.6	−0.1	95.4	−0.2
6310	3.8	116.8	116.9	+0.1	116.7	−0.1
10000	4.0	140.7	(141.3)	(+0.6)	140.6	−0.1
15850	4.2	167.2	(169.4)	(+2.2)	167.4	+0.2
19950	4.3	181.8	(185.1)	(+3.3)	181.9	+0.1
25100	4.3997	197.2	(202.0)	(+4.8)	197.2	0.0

However, the Antoine equation is as good an approximation as Cox's empirical parabolic relation up to about $T_R = 0.85$. This is illustrated in table 1, which is based on S. Young's (58) best selected values for the boiling points of *n*-pentane at various pressures. (These data are further discussed in a later section.) The Antoine equation used:

$$\log P\ (\text{mm.}) = 6.8723 - 1078.4/(t + 234.1) \tag{35}$$

fits the data to well within the reported accuracy up to $T_R = 0.83$ (about 117°C.), but gives increasingly larger errors from this temperature to the critical. On the other hand, the fit of the following Cox-type equation:

$$\log \frac{P}{760} = A\left(1 - \frac{T_B}{T}\right) \quad (36)$$

where $\log A = 0.64673 + 0.1817(1 - T_R)(0.865 - T_R)$, and (37)

$$T_B = 309.1748°\text{K.}, \quad T_c = 470.3°\text{K.}, \quad T_R = T/T_c$$

is not only almost as good as the fit of the Antoine equation up to $T_R = 0.83$, but this goodness of fit persists right up to the critical point.

From a practical point of view it is much more convenient to use two Antoine equations over the entire liquid range rather than the Cox equation. For example, the upper-range equation:

$$\log P = 7.5287 - 1562/(t + 244) \quad (38)$$

provides an excellent fit to ±0.1°C. for the pentane data from 100°C. ($T_R = 0.79$) to the critical point. One great advantage of the Antoine equation over equations of a more complex nature, such as the Cox equation, is the ease with which the temperature may be computed from the pressure, without the necessity of a trial-and-error solution.

The Antoine equation does not fit highly precise vapor-pressure data exactly over a large temperature range. The discrepancies are small but appear to be somewhat periodic in nature. Since similar systematic errors have been noted for other three-constant vapor-pressure equations, the author believes that no three-constant equation can adequately and accurately represent the true and complex vapor pressure–temperature relation. It is possible that a major improvement to the goodness of fit of the Antoine equation for these highly precise data, at least for T_R less than 0.75, could be obtained by a careful study of the deviations from the Antoine equation and the subsequent derivation of a five-constant equation. This problem is outside the scope of the present paper.

VI. DETERMINATION OF CONSTANTS IN THE ANTOINE EQUATION

The determination of the best values of the constants in empirical equations is a tedious and time-consuming task. Although the methods described below for the Antoine equation are believed to be the most satisfactory available, they still require a considerable amount of time, even for a small set of data. Similar methods may be derived for other simple empirical equations. The three commonest methods of curve fitting—selected-point, graphical, and least-squares—are discussed in order.

A. *Selected-point method*

In the selected-point method, three widely spaced and apparently good points are picked from the data and used to set up three simultaneous equations, which are solved for A, B, and C. If required, the points may well be selected by making a trial plot of $\log P$ against $1/(t + 230)$. Convenient formulas for performing this procedure are shown below. Let

$$y = \log P$$

and use subscripts to denote the three separate points; then we can solve for C, B, and A in that order, as follows:

$$\frac{y_3 - y_2}{y_2 - y_1} \cdot \frac{t_2 - t_1}{t_3 - t_2} = 1 - \frac{t_3 - t_1}{t_3 + C} \tag{39}$$

$$B = \frac{y_3 - y_1}{t_3 - t_1}(t_1 + C)(t_3 + C) \tag{40}$$

$$A = y_2 + B/(t_2 + C) \tag{41}$$

The great disadvantage of the selected-point method is that it neglects a major and valuable portion of the data, and an examination of the deviations of the calculated values from the observed may well show that the wrong three points were chosen.

B(1). Rapid graphical method

The rapid method of obtaining the constants in the Antoine equation, which is described below, has been under development by the author for several years. It has proved to be very useful for rapid interpolations and extrapolations, and the results obtained are usually almost as good as those obtained by more elaborate least-squares methods.

Estimation of C: For many purposes C can be assumed to be about 230°C. or 382°F., but since an incorrect value of C will cause a systematic error in the application of the equation, it is often desirable to have some way of estimating it. Least-squares studies have shown that the fit is not too dependent on the value of C used, so for most practical purposes C need only be obtained to 5° or even 10°C.

Frequently some points of the data are known to a higher degree of precision than others, so that at the start we can assume that one of these points is free from error. This point will be denoted by t_0, P_0. Since it is assumed that this point fits the Antoine equation perfectly, then:

$$y_0 = \log P_0 = A - B/(t_0 + C) \tag{42}$$

It is easily shown that:

$$\frac{y - y_0}{t - t_0} = \frac{A}{t_0 + C} - \frac{y}{t_0 + C} \tag{43}$$

and

$$\frac{t - t_0}{y - y_0} = \frac{C}{A - y_0} + \frac{t}{A - y_0} \tag{44}$$

Either of these equations may be used to evaluate C. A plot of $(y - y_0)/(t - t_0)$ *vs.* y is linear if the Antoine equation holds. The value of C is obtained from the slope, since

$$\frac{dy}{d\left(\frac{y - y_0}{t - t_0}\right)} = -(t_0 + C) \tag{45}$$

Similarly, using equation 44, the ratio of the intercept of the best straight line through $(t - t_0)/(y - y_0)$ *vs.* t to the slope gives the value of C.

It is usually desirable to plot "uncertainty bands" instead of points in applying equations 43 and 44. The uncertainty band is obtained by using the extreme uncertainties in t and P to obtain limits for each ratio, such as $(y - y_0)/(t - t_0)$. For example, suppose we have the following data: P is reported to be accurate to the nearest 0.1 mm., and t to the nearest 0.1°C. P_0, t_0 is taken to be exact.

$$\begin{array}{ll} P_0 = 760 \text{ mm.} & t_0 = 80.0°\text{C.} \\ P = 50 \text{ mm.,} & t = 11.8°\text{C.,} \\ \text{etc.} & \text{etc.} \end{array}$$

Let us consider the maximum value of $(y - y_0)/(t - t_0)$ for this first point.

$$\frac{y - y_0}{t - t_0} = \frac{\log 49.9 - \log 760}{11.9 - 80.0} = 0.01737$$

Similarly, the minimum value is 0.01729. The straight line or band connecting these points is plotted with the abscissa of $\log 50 = 1.699$. Similar bands are plotted for the other P,t points. The uncertainty in the ratios and the corresponding width of the bands increases as t approaches t_0.

The best straight line through these bands is drawn by eye, and gives the value of C, which should be rounded off to the nearest unit. It is interesting to note that by drawing the two extreme lines on the plot which will pass through most of the bands we can obtain the extreme values of C which will "fit" the data within the expected uncertainty.

The value of C so obtained may be checked by repeating the calculation with a new base point. This is usually not necessary, since the uncertainty in C is normally large enough to mask minor errors in t_0 and P_0.

Estimation of A and B: Once a good value of C has been obtained, several methods are available for estimating the values of A and B.

The first method does not require much time and is particularly useful when considerable faith can be put in the base point. For each point, B is computed using the relation:

$$B = \left(\frac{y - y_0}{t - t_0}\right)(t + C)(t_0 + C) \tag{46}$$

Since $(y - y_0)/(t - t_0)$, or its reciprocal, has already been calculated and $(t_0 + C)$ is a constant, this calculation presents no difficulties. If B systematically increases with temperature, the value of C chosen is too small and a larger value should be taken. The B value obtained should be averaged, omitting obviously bad points, and then rounded, usually to the nearest unit. The value of A is conveniently obtained from the base point, using the same equation as for point 2 in the selected-point method.

The second method is considered to be a rapid and superior variation of the familiar scheme of plotting deviations from a reference line drawn through the data when log P is plotted against $1/(t + C)$. A preliminary estimate of B is obtained in any convenient way, as by the application of equation 46 to a point of the data. Let this value be denoted by B_1. The value of A_1 is calculated for each point, using the following equation:

$$A_1 = y + \frac{B_1}{t + C} \tag{47}$$

If A, B, and C denote the final values of the constants we are seeking, then

$$A_1 = B_1 \frac{A}{B} + \frac{B - B_1}{B} y \tag{48}$$

or

$$A_1 = \alpha + \beta y$$

If α and β are the best values of the intercept and slope of the straight line obtained when A_1 is plotted against y (or, conveniently A_1 *vs.* P on semi-log paper) then B can be obtained from the equation

$$B = B_1/(1 - \beta) \tag{49}$$

This value of B may be conveniently rounded off, and the rounded value is used in equation to give A.

$$A = \alpha B/B_1 \tag{50}$$

B(2). Example of graphical method

As an example of the method which has just been presented, the Antoine equation will be calculated for methylcyclopentane, using seven points from the data of Max Schmitt (46). The serial numbers in table 2 refer to the original data. Point No. 15 was chosen as the base point, since it was believed that the precision of measuring the temperature and pressure is somewhat greater at the higher temperatures.

A graph of $(t_0 - t)/(y_0 - y)$ against t shows that a reasonable fit to the values can be obtained by drawing a line through the two points, No. 1 and No. 13. The equation of the line is

$$\frac{t_0 - t}{y_0 - y} = 58.020 + 0.25189\,t$$

C is obtained from the slope and intercept and equals $58.020/0.25189 = 230.3$. This can be safely rounded to 230.

The provisional value B_1 is then calculated according to equation 46, using the data for either of the two end points (Nos. 1 and 13).

$$B_1 = (230 - 16.00)(230 + 76.65)/53.99 = 1215.5 \text{ and}$$

$$B_1 = (230 + 65.90)(230 + 76.65)/74.62 = 1216.0$$

A_1 is now calculated for each point according to equation 47, using $C = 230$ and $B_1 = 1216$, and the values obtained are plotted against P on semi-log paper (figure 1). The bands shown correspond to the same uncertainty limits as before (± 0.2 mm. in P, ± 0.02° in t). It is obvious that the value of B has been judiciously chosen, since there is no marked trend of the A_1 values with P. The

TABLE 2

Calculation by rapid graphical method

NO.	t	P	y	$t_0 - t$	$y_0 - y$	$\frac{t_0 - t}{y_0 - y}$
	°C.	*mm.*				
1	−16.00	16.8	1.22531	92.65	1.71595	53.99 ±0.18*
2	0.00	41.8	1.62118	76.65	1.32008	58.06 ±0.11
4	19.80	109.3	2.03862	56.85	0.90262	62.98 ±0.08
8	38.45	238.3	2.37712	38.20	0.56414	67.71 ±0.08
11	50.23	369.9	2.56808	26.42	0.37318	70.80 ±0.10
13	65.90	626.9	2.79720	10.75	0.14406	74.62 ±0.22
15	76.65	873.5	2.94126	0.00	0.00000	Indeterminate

* Uncertainty bands corresponding to the arbitrary, reasonable values ± 0.02° in t and ±0.2 mm. in P.

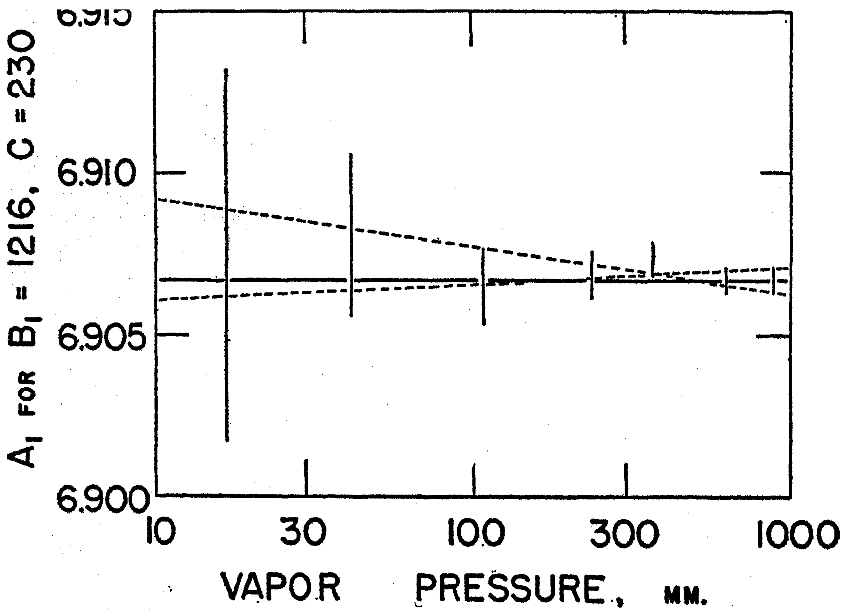


FIG. 1. Graphical determination of A and B

two broken lines shown in figure 1 represent the extreme values of α and β, and hence of A and B, which fit the data. Their equations are:

$$A_1 = 6.91067 - 0.00147y \text{ and} \tag{51a}$$

$$A_1 = 6.90560 + 0.00050y \tag{51b}$$

whence from equation 51a $B = 1214.2$, $A = 6.90044$; and from equation 51b $B = 1216.6$, $A = 6.90901$. The solid line represents a good compromise between these, with the value of B again rounded off to 1216. It corresponds to $\beta = 0$ and $\alpha = A = 6.90669$, so that the final equation is:

$$\log P = 6.90669 - 1216/(t + 230) \tag{52}$$

The fit is well within the reported precision with one somewhat poor point at 50.23°C. This example is only suitable for illustrative purposes, since but seven of the fifteen experimental points have been used. Actually, the fit is just as good for the other eight.

An alternative and equally suitable plan is to obtain A first, rather than B. After obtaining C by the procedure outlined above, a preliminary value of A (denoted by A_2) is obtained by applying equation 47 to any convenient point of the data preferably not close to the base point. B_2 is then calculated for each point of the data, using

TABLE 3

Comparison of results by rapid graphical method

NO.	t			P		
	Observed	Calculated	Deviation	Observed	Calculated	Deviation
	°C.	°C.	°C.	mm.	mm.	mm.
1	−16.00	−15.97	+0.03	16.8	16.8	0
2	0.00	0.06	+0.06	41.8	41.7	−0.1
4	19.80	19.79	−0.01	109.3	109.3	0
8	38.45	38.46	+0.01	238.3	238.2	−0.1
11	50.23	50.27	+0.04	369.9	369.3	−0.6
13	65.90	65.90	0	626.9	626.9	0
15	76.65	76.65	0	873.5	873.5	0

$$B_2 = (t + C)(A_2 - y) \tag{53}$$

and the values are plotted against t or $t + C$. It is easily shown that A and B, the final values of the constants we are seeking, are related to the preliminary values by the equation:

$$B_2 = B - (A_2 - A)(t + C) \tag{54}$$

and are, accordingly, readily obtained from the slope and intercept of the best line through the plotted values of B_2 and $t + C$.

It should be noted that these methods make no assumption as to the nature of the errors, whether in t or P or both. If t were given free from error, then only uncertainties in P should be considered for the bands. Similarly, if P were given free from error.

Empirical estimation of C: It would be very desirable if a general correlation of the value of C with some common physical property of the compound could be obtained. Such a correlation has eluded the author, but the following *approxi-*

mate rules have proved of utility, since they predict C to within 20° C. in most instances.

Rule 1: For hydrocarbons with n carbon atoms,

$$C = 271 - 7.6n \tag{55}$$

Rule 2: For elements with monatomic vapors and also for substances boiling below −150°C.,

$$C = 264 - 0.034t_B \tag{56}$$

where t_B is the normal boiling point in °C.

Rule 3: For all other compounds,

$$C = 240 - 0.19t_B \tag{57}$$

It is better, however, to determine the value of C from the data, even if they are not very reliable, since an incorrect value of C will cause an undesirable systematic error in the application of the equation.

C. Determination of constants by method of least squares

For very precise vapor-pressure data a better fit may be desired than the one obtained by the graphical method. This can be accomplished by the method of least squares. Unfortunately, although the method provides the most exact way of treating vapor-pressure data, the labor of computation is considerable even with an automatic calculator, and the method often cannot be applied rigorously because of the incomplete information accompanying the data. It is therefore usually not worth the time and trouble to apply least squares, since for most purposes just as satisfactory a solution for the constants can be obtained by the graphical method previously described.

The theory and computational details of least-squares procedures are very clearly and completely set forth in *Statistical Adjustment of Data*[3] by W. Edwards Deming, a revision of his earlier treatise, *Some Notes on Least Squares.* It is therefore not proposed to give a worked-out example of the application to the Antoine equation in detail,[4] although it is of pertinent interest to point out some of the advantages of such least-squares solutions in general.

Least squares may be considered to be based on three assumptions: (*1*) that the given type of equation is a true representation of the observations, (*2*) that the observations differ from the "true" values only by random or fortuitous errors, and (*3*) that these errors are normally distributed. If the degree of error differs from point to point, because of the experimental conditions, a system of weighting has to be imposed on the data. This system has to be based on the methods by which the data were obtained.

Least squares provides a very satisfactory method of curve fitting which gives reliable values of the empirical constants regardless of the form in which the equation is handled. The results are unique since, once the principles are established, the method always gives the same final results.

---

[3] Published by John Wiley and Sons, Inc., New York (1943).

[4] Such a worked-out example, including a "cookbook" procedure for applying least squares to vapor-pressure equations, may be obtained from the author.

Least squares also provides a method of adjusting the data to obtain the most probable values of the observational points, subject to the conditions which are imposed on the data. This process of adjustment may be considered as the determination of the most probable values of the residuals (experimental errors) by which the observed values differ from the true relation which we are seeking.

The method also provides a means of measuring the precision of the data, as measured by the goodness of fit, and also the precision of the values of the empirical constants obtained. Another convenient result is the precision (in terms of standard deviation and confidence limits) of the calculated values of either variable. We cannot assign a probability interpretation to values of the empirical constants or to measures of the variability of the data, if the method of least squares is not used.

It is to be noted that although the method of least squares, like any other mathematical treatment for fitting the equation to a single set of data, cannot detect systematic errors, a measure of the precision of a given set of data permits a comparison with the results of other sets of data on the same compound. This comparison does provide a means of determining systematic errors which is similar to the analysis of variance widely used in statistical work.

D. Systematic errors

Before leaving the subject of curve fitting it is well to point out that none of the curve-fitting procedures described can detect most kinds of systematic errors. An exception is the detection of the presence of inert gas in measurements of vapor pressures over a wide pressure range (11). If precise results cannot be fitted to an Antoine equation because of systematically increasing deviations at the lower pressures, the presence of inert gas should be suspected. A convenient mathematical treatment of the data is as follows: Take a suitable base point near the top of the temperature range involved (but not more than $T_R = 0.75$ to 0.80, the upper limit of the range of applicability of the Antoine equation) and calculate a preliminary Antoine equation, by the graphical method, for the high-temperature data. Extrapolate to the lower temperatures and estimate the partial pressure of the inert gas from the deviations at the lowest pressures. This partial pressure should be subtracted from the vapor pressures, making allowance for the expansion of the inert gas with temperature. Two further trials using the successively corrected vapor pressures should remove the systematic trend. Table 4 shows the results of the first and last corrections for a group of nine vapor-pressure measurements on hexamethylethane (11). The final adjusted values show a very high precision indeed.

VII. COMBINATION OF SETS OF VAPOR-PRESSURE DATA

A. Procedure

If the data of two or more reputable observers on the same compound disagree, then one or both sets are in error and should be discarded. Unfortunately these sets may be the only available information and a compromise equation is desirable until more reliable data are obtained. The procedure below for obtaining a compromise equation is a reasonable one, based on the fact that the presence of small amounts of impurities has but little effect on the heat of vaporization and hence affects the B constant of the Antoine equation but slightly. The same C constant is assumed to apply to all the sets, so that the burden of the systematic error falls on the A constant. In view of the fact that the sets of data are discrepant, the least-squares procedure is not applicable and a modification of the graphical method described above will be employed.

TABLE 4

Correction of systematic error due to inert gas

t	OBSERVED	CALCULATED[a]	DEVIATION	FIRST CORRECTION[b]	CORRECTED[c]	CORRECTED[d]	CALCULATED[e]	DEVIATION[f]
°C.								
4.89	7.0	6.0	−1.0	1.0	6.0	5.6	5.6	0.0
10.38	9.5	8.6	−0.9	1.0	8.5	8.1	8.2	+0.1
20.90	17.4	16.7	−0.7	1.0	16.4	15.9	16.0	+0.1
34.90	37.7	37.2	−0.5	1.1	36.6	36.2	36.1	−0.1
44.88	62.9	62.6	−0.3	1.1	61.8	61.2	61.3	0.0
59.97	128.7_5	128.7_7	0.0	1.2	127.55	127.0_5	127.0	0.0
79.96	300.6	300.7	+0.1	1.2	299.4	298.8	298.8	0.0
95.00	531.9	531.8_5	0.0	1.3	530.6	530.1	530.1	0.0
98.98	613.2	613.2	0.0	1.3	611.9	611.3	611.4	+0.1

[a] Calculated from the Antoine equation through the four highest points:

$$\log P = 7.8960 - 1701/(t + 234)$$

[b] First correction, by assuming 1 mm. at 10° or $0.0035T$ mm.

[c] First corrected values, by subtracting $0.0035T$ mm. from observed values.

[d] Last corrected values, by subtracting $0.0050T$ mm. from observed values.

[e] Calculated from the final Antoine equation through the corrected values:

$$\log P = 7.78882 - 1625.7/(t + 226)$$

[f] Deviation from final Antoine equation of the final corrected values.

The procedure is first to find an average Antoine C value using the graphical method on each set of data. A rough equation using this C and any convenient A and B is then used with each set for computing deviations in log P (as uncertainty bands), which are plotted against $1/(t + C)$, preferably on separate sheets to avoid confusion. The best values of B and their probable outside limits as judged by eye are then inspected and a suitable average value is chosen. The averaging of B and C is a purely arbitrary process, since the data possess unknown systematic errors which invalidate the usual statistical procedures. It is suggested that approximate weights be assigned on the basis of the goodness of fit of the line and perhaps the repute of the observer. The value of A can be obtained from a reliable boiling point, or from a suitable average point of the data.

B. Application to data for carbon disulfide

These principles are illustrated by application to the vapor-pressure data on carbon disulfide.

The precise data of Zmaczynski (59) over the range 30° to 85°C. were treated by least squares in a separate study and the following equation was found to fit the values to within about 0.003°C.:

$$\log P = 7.02814 - 1219.68/(t + 247.826)$$

H. von Siemens (47) made six determinations between −78° and 46°. These give a C value around 244, in excellent agreement with the 248 obtained for the Zmaczynski data. An average C value of 246 has been taken accordingly. These two sets of data are the only ones extensive enough to give reliable values of C and B. Using $C = 246$, we obtain values of B of 1205.5 from the Zmaczynski data and about 1211 from the von Siemens data. An average value of 1208

TABLE 5

Vapor pressure of carbon disulfide

t	P			SOURCE
	Observed	Calculated	Deviation	
°C.	*mm.*	*mm.*	*mm.*	
29.942	433.6	432.9	+0.7	Four points from Zmaczynski data (59)
46.262	760.0	760.0	0.0	
62.925	1268.1	1269.9	−1.8	
85.646	2347.4	2353.2	−5.8	
−78.19	0.68	0.65	+0.03	H. von Siemens (47)
−42.59	11.81	11.89	−0.08	
−21.52	42.69	42.93	−0.24	
0.00	127.00	127.15	−0.15	
19.67	294.27	293.16	+1.11	
46.25	760.00	759.70	+0.30	
−25.35	34.3	34.7	−0.4	Henning and Stock (25)
0.0	127.25	127.15	+0.1	
11.34	211.3	210.7	+0.6	
0.32	128.5	128.8	−0.3	M. Roland (44)
15.0	243.0	243.1	−0.1	
15.5	247.8	248.1	−0.3	N. L. Joukovsky (26)
18.0	274.6	274.4	+0.2	
19.3	290.1	288.9	+0.1	
20.5	303.1	302.9	+0.2	
22.8	331.1	331.2	−0.1	
24.3	350.3	350.7	−0.4	

is taken and, together with the good boiling point of Zmaczynski (46.262), gives the final compromise equation:

$$\log P = 7.01409 - 1208/(t + 246) \tag{59}$$

The values from this equation are compared in table 5, not only with the data above but also with other data which were not extensive enough to provide good values of B and C. The compromise equation is seen to be a good representation of the data from −78° to 85°.

C. Extrapolation to the critical point

By and large, the combination of sets of data requires not only a good deal of judgment but also a special technique for almost every problem handled, although the basic principles remain the same. As an example of such a special method which is very useful on occasion, details are given of the extrapolation of data taken at a lower temperature to the critical point, which is known. Strictly speaking, the Antoine equation provides a reliable fit only up to a reduced temperature of about 0.80, but the error is not serious at the upper range, especially when the equation passes through the critical point. For this type of calculation the deviations caused by this somewhat unwarranted use of the Antoine equation are of a secondary nature compared with the other uncertainties involved.

Suppose that we need an equation from 85°C. to the critical for carbon disulfide. For the moment we shall ignore the fact that there are data available in this range and simply use the information that the critical point is at 273°C., 75 atm.

A reasonable approach to the problem is to make the equation pass through the critical point and through a second point calculated from the known low-range equation at the highest temperature of the available data, and to have the slopes (dP/dt) equal at this second point. This gives three conditions enabling the three unknown Antoine constants to be determined.

Let A, B, C be the constants of the Antoine equation sought, and let A', B', C' be the constants of the known equation fitted to the low-range data. Also let t_1, P_1 be the highest temperature and calculated pressure of the low-range data and let t_c, P_c be the critical temperature and pressure. As above, let $y = \log P$.

The conditions can be stated as

$$A' - \frac{B'}{t_1 + C'} = y_1 = A - \frac{B}{t_1 + C} \tag{60}$$

$$\log P_c = y_c = A - \frac{B}{t_c + C} \tag{61}$$

$$\frac{B'}{(t_1 + C')^2} = \frac{B}{(t_1 + C)^2} \tag{62}$$

First, C is obtained from the equation:

$$\frac{B'}{(t_1 + C')^2} \cdot \frac{t_c - t_1}{y_c - y_1} = 1 + \frac{t_c - t_1}{t_1 + C} \tag{63}$$

then B from the relation:

$$B = \frac{y_c - y_1}{t_c - t_1} \cdot (t_1 + C)(t_c + C) \tag{64}$$

and A from equation 60, checked by the value from equation 61.

In the carbon disulfide example, $A' = 7.01409$, $B' = 1208$, and $C' = 246$; $t_1 = 85°$ and $\log P_1$ (mm.) $= 3.36454$; $t_c = 273°$ and $\log P_c$ (mm.) $= 4.75587$.

The following equation is obtained after suitable rounding of the constants:

$$\log P \text{ (atm.)} = 4.7253 - 1633/(t + 300) \tag{65}$$

TABLE 6

Example of extrapolation to the critical point

	I.C.T.	Equation 65	Deviation
°C.	*atm.*	*atm.*	*atm.*
100	4.42	4.39	−0.03
150	12.4	12.49	+0.09
200	28.3	28.8	+0.5
230	43.4	44.1	+0.7
260	64.1	64.5	+0.5
273 (critical)	75	75.0_5	0.0

A comparison with six points from the high-temperature data correlated in the *International Critical Tables* is shown in table 6. When it is recalled that equation 65 was derived from only the critical point and an extrapolation above 85° of a compromise equation and that the *International Critical Tables* lists twelve references as the basis for their correlation, the agreement is surprisingly good.

VIII. ANTOINE EQUATIONS DEVELOPED FOR CERTAIN VAPOR-PRESSURE DATA FROM THE LITERATURE

A. General considerations

In the course of this investigation over sixty Antoine equations have been derived. These range from approximate equations based on scanty data to careful least-squares studies on data of high precision. Many of them were obtained by earlier methods which are inferior to the direct ones outlined above. However, all provide a reasonable fit to the experimental values and, except for some highly precise data, most of them fit within a plausible estimate of the experimental error.

At this point, it should be noted that most experimenters have a higher opinion of the accuracy and reproducibility of their measurements than critical examination justifies. Even for a well-known compound like benzene, the discrepancies between competent observers are startling. The vapor pressure at 80°C. has been reported as 750 (Kahlbaum (27)), 751.86 (Regnault (43)), 753.62 (Young (56)), 755.0 (Young (57)), 756.4 (Schmitt (46)), 756.44 (Dejardin (18)), 757.12 (Zmaczynski (59)), 575.5 (Smith and Menzies (48)), 757.72 (E. R. Smith (50)), 757.80 (Smith and Matheson (51)), 759.7 (Nagornov (36)), 760 (Neubeck (39)), 764.5 (Mangold (33)), and 773.64 (Woringer (55))! Obviously several types of errors must be responsible, although it is usually impossible to assign a definite cause for even a large difference, when the work has been apparently carefully carried out. In the light of such discrepancies we should not take equations developed from the data of even reputable observers too seriously. The best we can do is to treat each set as carefully as possible, so that the equation developed represents a reasonable smoothing of the observer's values without the introduction of systematic bias from the equation employed.

In considering the goodness of fit of an empirical equation the nature of the residuals is as important as their magnitude. Bowing of the data, caused by the values being high in the ends but low in the middle or *vice versa,* is especially undesirable, since such a condition causes large errors in extrapolation. Every effort has been made to avoid bowing in the equations which have been developed.

Comparisons of the Antoine equation with the familiar $1/T$ form and with some of the other equations given in the literature are now presented for several representative compounds, followed by a tabulation of some of the Antoine equations which have been obtained during this investigation.

B. Comparison of Antoine and 1/T equations

It is not necessary to make extensive comparisons of the Antoine equation and the $1/T$ form, since it is evident that if vapor-pressure data are satisfactorily fitted by the Antoine equation, as is shown above, then the use of the $1/T$ equation will lead to systematic errors, unless the Antoine equation happens to have C equal to, or close to, 273. The examples were chosen to illustrate cases where C was close to 273 and quite far from it.

Isobutene: The recent data by Beattie, Ingersoll, and Stockmeyer (5) on isobutene enable a comparison to be made of their published "$1/T$ form" vapor-pressure equation and an Antoine equation derived from the experimental values (see table 7). The latter equation was obtained graphically and is a better fit than their equation, not only from the viewpoint of the smaller magnitude of the residuals, but also in that the Antoine residuals do not show the systematic bowing which is so markedly evident in the residuals from their $1/T$ equation.

International Critical Tables equations: The I.C.T. employs the $1/T$ equation as an interpolation equation for many of its vapor-pressure data of lower precision. The form used, in their notation, is as follows:

$$\log P = \frac{0.05223A}{t + 273.1} + B \qquad (66)$$

where A = average heat of vaporization in joules per gram-mole and B = constant (similar to the usual A of the Antoine formula). A few comparisons will be made of this equation and the Antoine equation, where the original data are readily available.

TABLE 7

Vapor pressure of isobutene

t	P	RESIDUALS IN P	
		$1/T$*	Antoine†
°C.	atm.	atm.	atm.
0	1.301	−0.007	+0.001
30	3.444	−0.009	−0.005
50	5.976	+0.010	+0.008
75	10.86	+0.04	+0.03
100	18.08	−0.04	−0.03
125	28.41	−0.03	+0.03

* Published equation: $\log P = 4.37592 - 1163.34/(t + 273.13)$.

† Antoine equation: $\log P = 4.32423 - 1128.4/(t + 268)$.

TABLE 8

Methyl ether

OBSERVED		RESIDUALS, OBSERVED — CALCULATED		
t	P	I.C.T.	Antoine	
°C.	mm.	mm.	mm.	°C.
−66.9	77.7	+0.5	−0.2	0.0
−63.8	95.2	+1.0	0.0	0.0
−60.0	119.6	+0.3	−1.1	+0.2
−52.4	189.2	+2.6	0.0	0.0
−46.6	261.7	+4.4	+0.7	−0.1
−41.4	326.2	−12.4	(−16.9)	(+1.0)
−37.3	426.6	+9.8	+4.7	−0.2
−30.9	524.0	−44.4	(−50.0)	(+1.9)
−28.5	639.4	+3.5	−2.0	+0.1
−27.2	679.2	+4.1	−1.4	+0.1
−26.3	711.5	+8.1	+2.7	−0.1
−25.3	745.0	+9.0	+3.8	−0.1
−24.1	781.7	+4.9	0.0	0.0

Methyl ether: The I.C.T. equation is:

$$\log P \text{ (mm.)} = 7.720 - 1202.6/(t + 273.1)$$

The experimental values (31) used to determine the equation and the goodness of fit of the Antoine equation:

$$\log P \text{ (mm.)} = 7.4119 - 1066/(t + 260)$$

are shown in table 8. It should be noted that the temperature measurements are evidently the controlling factor in the measurements, since 0.1°C. corresponds to from 0.5 mm. (at −67°C.) to 3.4 mm. (at −24°C.). The temperatures are reported to the nearest tenth of a degree and, except for the two bad points, the Antoine equation gives a satisfactory fit to within ± 0.2°C. The poorer fit of the I.C.T. equation is caused not so much by the incorrect choice of the value of C, since the data are not sufficiently precise enough to determine C accurately, but rather by poor judgment in fitting the $1/T$ equation. Probably too much weight was given to the two bad points.

Lead chloride and bromide: A comprehensive study of the literature on the vapor pressure of these compounds was made in this Laboratory in 1941 and showed that the following equations fit the data to within the experimental error:

$$PbCl_2\text{:}\quad \log P \text{ (mm.)} = 7.212 - 4340/(t + 48)$$

$$PbBr_2\text{:}\quad \log P \text{ (mm.)} = 7.233 - 4400/(t + 95)$$

TABLE 9

Vapor pressure of lead chloride and of lead bromide

t	P ($PbCl_2$)			P ($PbBr_2$)		
	I.C.T.	Antoine	Deviation	I.C.T.	Antoine	Deviation
°C.	*mm.*	*mm.*	*per cent*	*mm.*	*mm.*	*per cent*
500	0.24	0.20	+20	1.23	0.69	+78
600	3.0	3.3	−9	10.1	8.0	+26
700	22	26	−15	54	50	+8
800	113	124	−9	209	207	+1
900	440	430	+2	646	647	−0.2
1000	1380	1180	+17	1670	1640	+2

The Antoine C constants are widely different from 273, so that a considerable discrepancy is to be expected between these values and the I.C.T. equations:

$$PbCl_2\text{:}\quad \log P \text{ (mm.)} = 8.961 - 7411/(t + 273)$$

$$PbBr_2\text{:}\quad \log P \text{ (mm.)} = 8.064 - 6163/(t + 273)$$

The values from the two equations calculated at suitable intervals are shown in table 9, together with the percentage differences between them. Since the Antoine equation curves pass among sixty-three points by five observers (20, 23, 32, 53, 54) for lead chloride and twenty-seven points by three observers (23, 53, 54) for lead bromide, the fit is considered to be entirely adequate and the equations are considered to be a satisfactory representation of the data. The discrepancies of the I.C.T. equations are certainly outside any reasonable estimate of the experimental error.

Conclusions concerning 1/T equations: The indiscriminate use of $1/T$ equations is not recommended. For compounds boiling below room temperature the results may be quite satisfactory, since preliminary studies have indicated that for these compounds the value of C is usually greater than 250. It is suggested, for such low-boiling compounds, that the $1/T$ equation be tried and the deviations in log P examined by plotting them against $1/T$. If a distinct bowing of the points is evident, the use of $1/T$ is introducing a systematic error and an Antoine equation should be used instead. If, however, no bowing is noticed and the deviations appear to scatter randomly about a straight line, then the $1/T$ equation is quite satisfactory.

C. Comparison with Nernst equation, for hydrogen chloride

The following can be taken as a good example of data of fairly high precision. The authors (Giauque and Wiebe (22)) compared their data with the Nernst equation of Henning and Stock:

$$\log P \text{ (mm.)} = 4.65739 - 905.53/T + 1.75 \log T - 0.005077T$$

The following Antoine equation can be fitted to the data:

$$\log P \text{ (mm.)} = 7.16323 - 743.3/(T - 14.5)$$

TABLE 10

Vapor pressure of hydrogen chloride

OBSERVED		RESIDUAL IN t		RESIDUAL IN P	
T	P	Antoine	Nernst	Antoine	Nernst
°K.	mm.				
158.91	103.71	+0.01	−0.02	−0.06	+0.14
164.62	162.9	0.00	−0.03	0.00	+0.3
169.28	229.8	−0.01	−0.03	+0.2	+0.5
173.95	317.1	+0.01	0.00	−0.3	−0.1
178.53	428.1	+0.01	+0.01	−0.2	−0.2
181.84	526.4	0.00	0.00	−0.1	−0.1
185.17	643.4	−0.02	−0.01	+0.6	+0.5
188.41	774.8	0.00	0.00	0	+0.1
191.96	943.9	−0.01	−0.02	+0.5	+1.1
195.93	1165.1	0.00	−0.02	+0.1	+1.8

The goodness of fit of both equations is shown in table 10. The residuals are computed, as usual, by subtracting the calculated value from the observed. In these measurements the temperature has evidently a greater effect on the precision than the pressure. The Nernst equation shows more bowing than the Antoine, so is much less safe for extrapolation. For example, on extrapolation to the critical pressure (81.55 atm., a fortyfold change in pressure), the Antoine equation gives 54.9°C., only 3.5° higher than the correct value of 51.4°C., whereas the Nernst equation gives 88.0°C., almost 40°C. too high. (This value has to be obtained by a trial-and-error process, since the Nernst equation cannot be solved directly for t.)

The dangers in extrapolating equations of this type become more obvious when the value of d log P/dT is considered. The equation can be written

$$\log P = y = A - \frac{B}{T} + D \log T + ET \tag{67}$$

whence

$$\frac{dy}{dT} = \frac{B}{T^2} + \frac{D}{2.3T} + E = \frac{1}{T}\left(\frac{B}{T} + \frac{D}{2.3} + ET\right) \tag{68}$$

When several constants of an empirical equation have to be obtained from a set of data there is often a fairly wide range of values which they can be given which will fit the data with almost the same precision. In least-squares work this is termed instability. However, values of the first derivative outside of the range studied may be very sensitive to these changes in the constants, so much so that the calculated vapor pressure may actually decrease with temperature. Thus we note that the signs of the terms in B and E have been changed, so that while small differences in these constants may cancel out in equation 67, these differences are cumulative in equation 68. A similar line of reasoning may be applied to the two other pairs of constants.

D. Comparison with Biot equation, for heptane and octane

Sidney Young (57) fitted his long-range vapor-pressure data by means of the Biot equation, which contains five empirical constants. At least up to a reduced temperature of 0.85, a fit as good or better can be obtained with the Antoine equation. For *n*-heptane, the Antoine equation fits with a standard deviation of 0.27 per cent in P from 0° to 220°C. (the critical point is at 266.85° C.). This is a better fit than Young's Biot equation. Similarly, for *n*-octane the fit is about as good as the Biot equation from 10° to 230°C., with a standard deviation of 0.65 per cent in P.

E. Comparison with data smoothed by cross plotting, for the normal paraffin hydrocarbon series

In 1928 Sidney Young (58) made a critical compilation of the available vapor-pressure data on the normal paraffin hydrocarbons. Convenient pressure intervals between 11 and 19,950 mm. were chosen, and a cross-check was obtained by considering the boiling points at these pressures as a function of n, the number of carbon atoms. The following empirical equation was fitted to the temperatures at each pressure:

$$\log T = a + b \log n + c(\log n)^2 + d(\log n)^3 \tag{69}$$

The cubic term was only required at the three lowest pressures: 11, 15, and 30 mm.

Young also found that the boiling-point increment equation,

$$\Delta = A/T^{B\sqrt{T}} \tag{70}$$

for the change in boiling point (Δ) for each addition of the CH_2 group, could be generalized to pressures other than 760 mm. The values of A and B, respectively, range from 92.6 and 0.0159 (at 11 mm.) to 251 and 0.0133 (at 19,950 mm.).

A third cross-check on the data was afforded by the Ramsay–Young rule, using hexane as the reference compound. If T_A, T_B represent the absolute boiling points of the two compounds at the same pressure, then the relationship can be formulated as:

$$T_A/T_B = a + bT_A + c(T_A)^2 \tag{71}$$

where a, b, c depend on the pressure and the compound under comparison.

Using reliable observational data and the three methods above for intercomparison, Young obtained what he considered the most probable boiling points for pressures ranging from 11 to 19,950 mm. for each member of the series from methane to heptane, from 11 to 15,850 mm. for octane, from 11 to 760 mm. for nonane to nonadecane, and at 11, 15, and 30 mm. for the higher normal paraffins from $n = 20$ to 35. This set of data affords an excellent opportunity for determining the systematic variation of the Antoine constants. Antoine equations were accordingly obtained by the usual graphical methods and their constants are given in table 11, together with the maximum deviations between the equations and Young's most probable boiling points. The individual deviations are of about the same size as or smaller than the differences between Young's selected observed values and his values by the three methods of intercomparison. It is believed that the Antoine equations presented are as good a representation of the true values as Young's tabulation.

TABLE 11

Vapor pressure of the normal paraffins, C_nH_{2n+2}

n	A	B	C	RANGE	RANGE OF DEVIATIONS
				mm.	°C.
1	6.421	352	261	11– 2000	0.0, +0.1
2	6.884	683	259	11–10000	−0.2, +0.4
3	6.919	862	256	11–10000	−0.3, +0.2
4	6.8857	986	247	11– 7000	−0.1, +0.2
5	6.936	1112	238	11–10000	−0.3, +0.2
6	6.910	1192	227	11– 7000	−0.2, +0.3
7	6.926	1284	219	11– 7000	−0.2, +0.1
8	6.9345	1360	210	11– 7000	−0.1, +0.2
9	6.9313	1429	203	11– 760	−0.2, +0.1
10	6.9355	1497	196	11– 760	−0.1, +0.1
11	6.978	1581	191	11– 760	−0.1, +0.2
12	7.051	1681	188	11– 760	−0.1, +0.2
13	7.0972	1758	183	11– 760	0.0, +0.2
14	7.0076	1744	170	11– 760	−0.2, +0.3
15	6.8552	1678	152	11– 760	−0.2, +0.2
16	6.688	1599	133	11– 760	−0.2, +0.2
17	6.545	1535	116	11– 760	−0.1, +0.1
18	6.474	1516	104	11– 760	−0.2, +0.2
19	6.408	1506	94	11– 760	−0.2, +0.4

In line with other physical properties of homologous series we could expect that the Antoine constants would fall on smooth curves when plotted against the number of carbon atoms. Actually, as has been pointed out in the discussion on instability above, the constants are sensitive to small variations, so that sets of constants which appear to be quite different actually provide about the same fit to the data. The values of C in the Antoine equations for the paraffins have been somewhat smoothed and fall on a fairly smooth curve with an abrupt inflection at $n = 14$. The vapor pressures of the higher members of the series are not known as precisely as those of the lower members and the intercomparison methods are not as satisfactory, so that it is difficult to determine whether a true inflection point exists at $n = 14$ or not. Since the values of A and B were obtained after C had been decided on, this inflection point is also reflected in the plots of A and B. The writer believes that the values of all three constants will fall on smooth curves when more accurate data are obtained. The small variation of A with structure is of especial interest.

The trend is clear that C increases as the boiling point decreases. The approximate correlation of C with boiling point (equations 55, 56, 57) was obtained from a large plot of these data and about fifty other values of C, obtained from the literature and by calculation, on substances ranging from elements to inorganic salts and a variety of organic compounds. For data of moderate precision, $C = 230$ appears to be a suitable average value for most organic compounds boiling between 0° and 150°C.

Some studies have shown that for the upper range of vapor-pressure data, say from a reduced temperature of 0.75 to the critical point, the value of C is considerably higher than for the lower range, being even higher than 273. A set of equations for this upper range could have been fitted to the paraffin data, but the data are not known to the same precision as for lower temperatures and the present set of equations was believed to be of more general interest in showing the variation of the value of C for the more useful range of temperatures and pressures.

It should be noted that temperatures calculated from the equations in table 11, above the ranges indicated will be a few degrees too high.

Recent work (54a) at the National Bureau of Standards on the vapor pressures of over fifty hydrocarbons from 48 mm. (or 15°, if the pressure at 15° is above 48 mm.) up to 780 mm. shows some minor differences from the results of table 11. This is to be expected, since the range is much shorter and the data are on pure compounds rather than correlated values.

F. Miscellaneous Antoine equations

The Antoine equations which are tabulated in this section were obtained in various ways, as has been pointed out. They have accordingly been divided into two groups (see tables 12A and 12B). In the first group all three Antoine constants have been determined. The equations for the following compounds, which have already appeared in the text, have not been included: isoöctane, (page 7); water (page 10); methylcyclopentane (page 15); carbon disulfide (page 23); isobutene (page 24); methyl ether (page 25); lead chloride (page 26); lead bromide (page 26); hydrogen chloride (page 27); normal paraffins (page 30).

TABLE 12A

Antoine equations: Group 1

COMPOUND	SOURCE OF DATA	*A*	*B*	*C*	*s*	RANGE
Pentane....................	Young[1]	7.5287	1562	302	±0.1°	100 to 197.2°C.
Neopentane..............	Whitmore, Fleming[2]	7.6061	1462	300	2.5 mm.; 0.25°	−42 to 9.4°C.
n-Heptane................	E. R. Smith[3]	6.89662	1264.663	216.494	0.045 mm.	40 to 125°C.
Toluene.....................	Max Schmitt[4]	6.92834	1335	219	0.42 mm.	14 to 89°C.
Toluene.....................	Schouteden, Deveux[5]	6.50924	1088.2	189	0.54 mm.	22 to 111°C.
Methyl chloride...........	Messerly, Aston[6]	7.0601	928.4	246.3	0.014 mm.	191 to 249°K.
Iron carbonyl..............	Trautz, Badstübner[7]	6.8251	1183.6	195	±0.5 mm.	25 to 91°C.
Sulfur dioxide	Giauque, Stephenson[8]	7.27886	998.673	237.1	0.17 mm.	197 to 263°K.
Aluminum chloride	Kelley[9]	5.760	669.2	71	±1 per cent in *P*	193 to 220°C.

[1] Sci. Proc. Roy. Dublin Soc. **12**, 374 (1910); excellent fit up to critical point.

[2] J. Am. Chem. Soc. **55**, 3803 (1933); good to ±3.5 mm., ± 0.5°C.

[3] J. Research Natl. Bur. Standards **24**, 229 (1940); equation shown is a better fit than the author's Antoine equation from the same data.

[4] Pub. sci. tech. ministère de l'air (France), **No. 54** (1934).

[5] Natuurwet. Tijdschr. **18**, 242 (1936).

[6] J. Am. Chem. Soc. **62**, 886 (1940); a very good fit except for two extreme deviations of −0.22 and +0.43 mm. The others range from −0.03 to +0.02 mm.

[7] Z. Elektrochem. **35**, 799 (1929). Their data went down to 67°. Some unpublished measurements by V. Hnizda of this laboratory checked the lower range and extended the range to 25°.

[8] J. Am. Chem. Soc. **60**, 1389–94 (1938); not as good a fit as their four-constant equation, which gives $s = 0.038$ mm.

[9] Kelley (U. S. Bur. Mines Bull. **383**, 17 (1935)) obtained a good fit using a Rankine-type equation up to 220° C., but his equation gives decreasing values of vapor pressure on extrapolation above 530°C. The Antoine equation shown fits Kelley's calculated values to within mm. and gives much more reasonable values on extrapolation to higher temperatures.

TABLE 12B

Antoine equations: Group 2

C = 230 in every case

COMPOUND	SOURCE OF DATA	*A*	*B*	*s*	RANGE
Benzene	M. Schmitt[1]	7.0720	1300	0.25 per cent in *P*	9 to 81°C.
Benzene	Compromise[2]	7.06664	1298		9 to 81°C.
Toluene	M. Schmitt[1]	7.1386	1449	0.25 per cent in *P*	13 to 89°C.
Hexane	Compromise[2]	6.96473	1220		0 to 80°C.
Cyclohexane	M. Schmitt[1]	6.9727	1272	0.16 per cent in *P*	28 to 81°C.
Methylcyclopentane	M. Schmitt[1]	6.9068	1216	0.16 per cent in *P*	−16 to 77°C.
Methylcyclohexane	J. C. Pope[3]	6.8101	1300	2.5 mm.	98 to 113°C.
Cyclobutane	Handbook[4]	6.6284	909		
Acetone	Handbook[4]	7.1625	1225		
Decalin	I.C.T.	7.2216	1824		
Cyclohexanol	Gardner, Brewer[5]	8.3956	2144	2.8 mm.	34 to 149°C.
Ethyl chloride	Compromise[2]	6.8994	975.3	±1 per cent	0 to 100°C.
B_2H_7N	Schlesinger *et al.*[6]	7.1579	1300	1.9 per cent in *P*	−27 to 29°C.
Diisopropylzinc	V. Hnizda[3]	7.987	1858	±0.5°, ±0.7 mm.	37 to 65°C.
Tetramethyllead	Callingaert *et al.*[7]	6.9381	1378.7	±10 per cent in *P*	25 to 760 mm.
Trimethylethylead	Calingaert *et al.*[7]	7.2760	1602.5	±10 per cent	13 to 100 mm.
Dimethyldiethyllead	Calingaert *et al.*[7]	7.5903	1810.7	±10 per cent	10 to 50 mm.
Methyltriethyllead	Calingaert *et al.*[7]	7.8768	2000.5	±10 per cent	4 to 15 mm.
Tetraethyllead	Calingaert *et al.*[7]	8.1547	2184.6	±10 per cent	0.05 to 300 mm.

[1] Pub. sci. tech. ministère de l'air (France), No. 54 (1943).

[2] The compromise equations were obtained from several sets of data, using the methods described above.

[3] Unpublished data from this laboratory.

[4] Approximate equations obtained from data tabulated in various handbooks.

[5] Ind. Eng. Chem. 29, 179 (1937).

[6] J. Am. Chem. Soc. 60, 2298 (1938); a slightly better fit than their Nernst equation.

[7] J. Am. Chem. Soc. 61, 2757 (1939). These equations all converge to 432°C. and log $P = 4.8566$ when log P is plotted against $1/(t + 230)$. This intersection point can be used for obtaining the vapor pressures of mixtures of these alkyls.

In the second group the value of C has been taken equal to 230. Many of these equations were obtained from data of lower quality than those in the first group.

A rough measure of the goodness of fit is afforded by an estimate of the standard deviation of the residuals in pressure or temperature. This has been denoted by s. In some instances the spread of the residuals has been shown preceded by a ± sign.

G. Vapor-pressure nomograph

An alignment chart can be easily constructed for the Antoine equation with C equal to 230. Such a chart, constructed using data from many sources, is shown in figure 2. The hydrocarbon line was drawn through the Calingaert and Davis intersection point of 1240°C. and 1.3×10^6 mm. (see above). The lead alkyl line was similarly drawn through the intersection point of 432°C. and 7.19×10^4 mm. For mixtures, or in the absence of other data, these lines can be used to estimate vapor pressures using a normal boiling point. For example, if a hydrocarbon mixture boils at 130°C., what is its vapor pressure at room temperature (25°C.)? The line from 760 mm. to 130°C. intersects the hydrocarbon line determining the vapor-pressure point. Use of this point and 25°C. gives a vapor pressure of 13 mm.

Often a good guess can be made of the vapor pressure of a compound by drawing a curve through the points corresponding to similar compounds and using this curve in a similar manner to the hydrocarbon line.

The chart can be used for interpolations and extrapolations by using two or more vapor pressures to determine the point on the nomograph.

IX. HEAT OF VAPORIZATION

The heat of vaporization is related to the vapor pressure by the Clausius–Clapeyron equation, which may be written in the form:

$$\Delta H = \Delta z \cdot RT^2 \frac{\mathrm{d} \ln P}{\mathrm{d}T} \tag{72}$$

where $\Delta z = P\Delta V/RT = PV_g/RT - PV_l/RT$. The calculation of the heat of vaporization thus resolves itself into two parts, the determination of the slope of the vapor-pressure curve and the determination or estimation of ΔV or Δz. When the Antoine equation is applicable, equation 72 becomes:

$$\Delta H = \frac{4.57566BT^2}{(T + C)^2} \Delta z \tag{73}$$

Similar expressions may be derived for other empirical vapor-pressure equations. If ΔV is known from P–V–T studies, the following equivalent form may be more convenient:

$$\Delta H = \frac{BPT\Delta V}{13629(T + C)^2} \tag{74}$$

P is in millimeters, t in °C., ΔV in cubic centimeters per mole, and ΔH in calories per mole.

Unfortunately, P–V–T data for both liquid and gaseous phases are not always available, but for many purposes a correlation of Δz with reduced temperature and pressure is entirely adequate. Probably the best available correlation is that of Meissner (34), whose figure 4 shows Δz as a function of P_R and T_R. A similar correlation (14) was obtained by plotting Δz against P_R/T_R for Young's data on n-pentane, isopentane, n-hexane, and n-heptane (57). The correlating curve is believed to be suitable for predicting ΔH to within ±5 per cent, at least for non-polar compounds, and has not proved to be far in error for polar compounds. The two equations below are equivalent to the correlation.

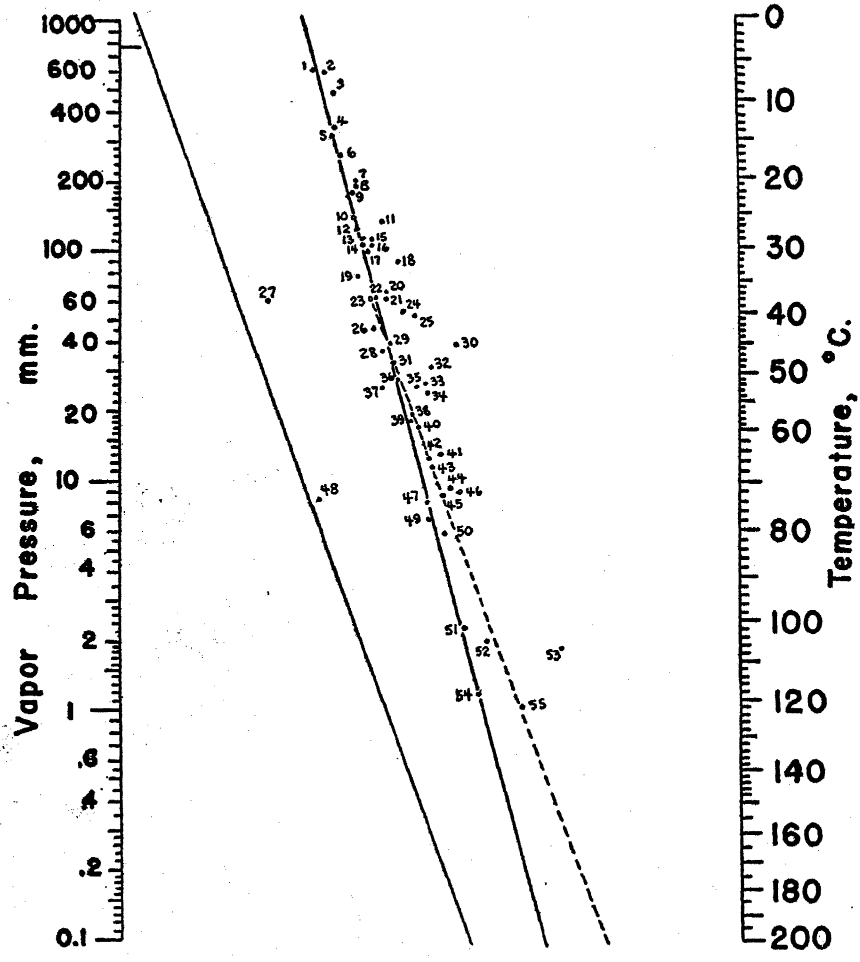


FIG. 2. Vapor-pressure nomograph. The dotted diagonal line applies to the homologous series of lead alkyls and the two solid diagonal lines to most hydrocarbons and their halogenated derivatives, the one at the right for pressure in millimeters as shown and the one at the left for pressure in *atmospheres*.

For P_R/T_R from 0 to 0.2,

$$\log \Delta z = 2.106x^2 - 1.0268x \qquad (75)$$

For P_R/T_R from 0.2 to 1.0,

$$\log \Delta z = 0.136x^2 - 0.645x - 0.0185 \tag{76}$$

where $x = -\log (1 - P_R/T_R)$.

For the useful range of values of P_R/T_R less than 0.10, the correlation is reliably represented by the simpler relation:

$$\Delta z = 1 - 0.97P_R/T_R \tag{77}$$

As an example of the use of these methods, the heat of vaporization of *n*-heptane is computed at the normal boiling point.

According to E. R. Smith (49) the normal boiling point is 98.428°C. and the vapor-pressure equation is:

$$\log P = 6.905113 - 1269.821/(t + 217.11)$$

Vapor-pressure points for figure 2

1 cyclobutane
13 benzene
13 cyclohexane
49 naphthalene
47 kerosene (refined No. 9 oil)

48 methyl bromide (atm. *not* mm.)
2 ethyl chloride
10 ethyl iodide
5 ethyl bromide
17 ethylene dichloride
28 ethylene dibromide
12 carbon tetrachloride
9 chloroform

11 methyl alcohol
18 ethyl alcohol
25 *n*-propyl alcohol
32 *n*-butyl alcohol
34 *n*-amyl alcohol
41 cyclohexanol
44 methylcyclohexanol

7 ethyl formate
15 ethyl acetate
20 ethyl propionate
16 propyl formate
21 propyl acetate

37 ETHYL Aviation Fluid
26 ETHYL Motor Fluid

24 water
53 aluminum chloride
46 solid iodine
27 hydrogen chloride (atm. *not* mm.)

8 acetone
3 acetaldehyde
4 ethyl ether

23 tetramethyllead
31 trimethylethyllead
38 dimethyldiethyllead
42 methyltriethyllead
45 tetraethyllead

29 trimethylaluminum
40 methylaluminum dichloride

43 triethylbismuth
50 tri-*n*-propylbismuth
51 tri-*n*-butylbismuth
54 tri-*n*-amylbismuth

39 diethylmercury
52 di-*n*-amylmercury
55 di-*n*-hexylmercury

36 trimethylgallium
33 trimethylindium
35 diisopropylzinc

19 iron pentacarbonyl
6 nickel carbonyl

6 aluminumborohydride (AlB_3H_{12})
30 berylliumborohydride (BeB_2H_8)

14 mono-*N*-methyltriborinetriamine
22 di-*N*-methyltriborinetriamine
5 tri-*N*-methyltriborinetriamine

The critical point is taken to be 540.17°K. and 27.00 atm. (6) so that:

$$P_R/T_R = (1/27.00)/(371.588/540.17) = 0.0538$$

From equation 77, $\Delta z = 0.946$ and from the more complicated equation 75, $\Delta z = 0.947$. Meissner's chart gives $\Delta z = 0.95$.

ΔH is calculated by equation 73 to be:

$$\frac{(4.57566)(1269.821)(273.16 + 98.248)^2(0.947)}{(217.11+98.428)^2} = 7630 \text{ calories per mole}$$

This is an excellent check on the calorimetric value, 7660 ± 20 calories per mole (41), which corresponds to a Δz value of 0.951 ±0.003.

TABLE 13

Comparison of Δz for a polar liquid: ethyl alcohol above the boiling point

TEMPERATURE	Δz	
	I.C.T.*	Correlation†
°C.		
80	0.97_5	0.97_5
100	0.95_0	0.95_2
120	0.91_2	0.91_7
140	0.86_8	0.86_8
160	0.81_3	0.81_0
180	0.73_3	0.73_3
200	0.62_0	0.62_7
220	0.46_8	0.47_8
230	0.37_3	0.37_5
240	0.17_9	0.22_9
243.1 (critical)	0.0	0.0

* Using the I.C.T. values for densities of liquid and vapor and for vapor pressure.

† Calculated from the correlation equations, 75 and 76.

For polar liquids the errors in z_g and z_l tend to compensate, so that the estimated value of Δz is not as much in error as would be expected. Table 13 shows the excellent agreement for ethyl alcohol between the correlation and values of Δz calculated from density and vapor-pressure values in the *International Critical Tables.* It is believed that the use of the Δz correlation presented above will not cause an error of more than ±10 per cent in the estimation of the heat of vaporization at any temperature and that in most instances the error will be less than 5 per cent. The error caused by the use of the Antoine equation, or any other reliable vapor-pressure equation, for computing the slope, $d \ln P/dT$, is believed to be a very minor factor in the calculation. However, the use of an unsatisfactory vapor-pressure equation will invalidate the method.

X. OTHER APPLICATIONS OF THE ANTOINE EQUATION

The Antoine equation may be used for several kinds of data other than vapor pressures of pure liquids.

Antoine (3) suggested its use for representing the temperature relation of the density of a saturated vapor, and Schmidt (45), apparently independently, proposed a similar scheme for representing the product of the densities of saturated liquid and vapor, or

$$\log (d_l \cdot d_g) = A - \frac{B}{t + C} \tag{78}$$

For *n*-heptane, the following equation gives an excellent fit to Young's data (57) up to 200°C. ($T_R = 0.88$):

$$\log (d_l \cdot d_g) = 1.1391 - \frac{1275.1}{t + 235.9} \tag{79}$$

where d_l, d_g are in grams per cubic centimeter.

The value of C and the range of applicability are similar to those obtained for vapor pressures.

A useful chart for the fugacities of liquid hydrocarbons was prepared by W. C. Kay (28), using a scheme similar to the Cox chart. It can be shown to be equivalent to the use of Antoine equations of the form:

$$\log f_l = A - B/(t + 180) \tag{80}$$

all passing through the intersection point of the family at 1054°C. and 367 atm. The fugacity of a hydrocarbon can be approximated by using these relations and the additional fact that at sufficiently low pressures, the fugacity becomes equal to the vapor pressure.

Ideal vapor–liquid vaporization constants, usually known as K values ($K = y/x$), vary with temperature somewhat as vapor-pressure data do. If Antoine equations are used for the temperature relation, the fit is usually as good as the number of significant figures reported.

The Antoine equation may also be used to represent the temperature variation of the kinematic viscosities of certain liquids. Thus, the following equation fitted unpublished data on redistilled commercial xylidine ($d_{4°}^{20°} = 1.5606$) to within the experimental error from −40° to 130°F.

$$\log KV = -0.912 + 290/(t + 117) \tag{81}$$

where KV is kinematic viscosity in centistokes and t is temperature, °F.

The Antoine equation is also useful for ideal relative volatility or vapor-pressure ratio data.

The author gratefully acknowledges the assistance of several members of the Chemical Research Laboratory staff of the Ethyl Corporation, in particular Drs. George Calingaert, Harold A. Beatty, and Augustine O. Allen, and Mr. Hymin Shapiro.

REFERENCES

(1) Antoine, C.: Compt. rend. **107,** 681 (1888).
(2) Antoine, C.: Compt. rend. **107,** 836 (1888).

(3) ANTOINE, C.: Compt. rend. **107,** 1143–5 (1888).

(4) ASHWORTH, A. A.: J. Inst. Petroleum Tech. **10,** 787–802 (1924).

(5) BEATTIE, J. A., INGERSOLL, H. G., AND STOCKMEYER, W. H.: J. Am. Chem. Soc. **64,** 546–50 (1942).

(6) BEATTIE, J. A., AND KAY, W. C.: J. Am. Chem. Soc. **59,** 1586–7 (1937).

(7) BIOT, J. B.: *Connaissance des temps,* 1844.

(8) BOSE, E.: Physik. Z. **8,** 944–51 (1907).

(9) CALINGAERT, G., BEATTY, H. A., AND NEAL, H. R.: J. Am. Chem. Soc. **61,** 2755–8 (1939).

(10) CALINGAERT, G., AND DAVIS, D. S.: Ind. Eng. Chem. **17,** 1287–9 (1925).

(11) CALINGAERT, G., SOROOS, H., HNIZDA, V., AND SHAPIRO, H.: J. Am. Chem. Soc. **66,** 1389–94 (1944).

(12) CARBONELLI, C. E.: Gazz. chim. ital. **49,** I, 151–3 (1919).

(13) CHWOLSON, O. D. (*Editor*): *Traité de physique,* Vol. 3, pp. 766–78. Paris (1910).

(14) *Cf.* COPE, J. Q., LEWIS, W. K., AND WEBER, H. C.: Ind. Eng. Chem. **23,** 887–92 (1931).

(15) COX, E. R.: Ind. Eng. Chem. **15,** 592–3 (1923).

(16) COX, E. R.: Ind. Eng. Chem. **28,** 613–16 (1936).

(17) DALTON, J.: Mem. Proc. Manchester Lit. & Phil. Soc. **5,** 550 (1801).

(18) DEJARDIN, G.: Ann. phys. [9] **11,** 253–91 (1919).

(19) *Cf.* DODGE, B. F.: *Chemical Engineering Thermodynamics,* 1st edition, pp. 252–7. McGraw-Hill Book Company, Inc., New York (1944).

(20) EASTMAN, E. D., AND DUSCHAK, L. H.: U. S. Bur. Mines Tech. Papers, **No. 225** (1919).

(21) GAMSON, B. W., AND WATSON, K. M.: Natl. Petroleum News **36,** R-258–64 (May 3, 1944).

(22) GIAUQUE, W. F., AND WIEBE, R.: J. Am. Chem. Soc. **50,** 101–22 (1938).

(23) GREINER, B., AND JELLINEK, K.: Z. physik. Chem. **165A,** 97 (1933).

(24) HENGLEIN, F. A.: Z. physik. Chem. **98,** 1 (1921).

(25) HENNING, F., AND STOCK, B.: Z. Physik **4,** 226–44 (1921).

(26) JOUKOVSKY, N. L.: Bull. soc. chim. Belg. **43,** 397 (1934).

(27) KAHLBAUM, G. W. A.: Z. physik. Chem. **26,** 603 (1898).

(28) KAY, W. C.: In T. K. Sherwood's *Absorption and Extraction,* 1st edition, pp. 101–2. McGraw-Hill Book Co., Inc., New York (1937).

(29) KELLEY, K. K.: U. S. Bur. Mines Bull. No. 383 (1935).

(30) KIRCHHOFF, G.: Ann. Physik **104,** 612 (1858).

(31) MAASS, O., AND BOOMER, E. H.: J. Am. Chem. Soc. **44,** 1709–28 (1922).

(32) MAIER, C. G.: U. S. Bur. Mines Tech. Papers, **No. 360,** p. 28 (1925).

(33) MANGOLD: Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse **102,** IIa, 1071 (1893).

(34) MEISSNER, H. P.: Ind. Eng. Chem. **33,** 1440–3 (1941).

(35) MILES, F. T.: Ind. Eng. Chem. **35,** 1052–61 (1943).

(36) NAGORNOV, N. N.: Ann. inst. anal. phys. chim. (U. S. S. R.) **3,** 562–83 (1927); Chem. Abstracts **22,** 4298^{6} (1928).

(37) NERNST, W.: Gött. Nachr. **1** (1906).

(38) *Cf.* NERNST, W.: *Theoretical Chemistry,* p. 234. Macmillan and Company, London (1916).

(39) NEUBECK: Z. physik. Chem. **1,** 649 (1887).

(40) OSBORNE, N. S., AND MEYERS, C. H.: J. Research Natl. Bur. Standards **13,** 1–20 (1934).

(41) PITZER, K. S.: J. Am. Chem. Soc. **62,** 1224–7 (1940).

(42) RANKINE, W. J. M.: Edinburgh New Phil. J. **94,** 235 (1849).

(43) REGNAULT: Mem. Paris **26,** 339 (1862).

(44) ROLAND, M.: Bull. soc. chim. Belg. **37,** 117 (1928).

(45) SCHMIDT, A.: J. chim. phys. **15,** 97–153 (1917).

(46) SCHMITT, MAX: Publ. sci. tech. ministère de l'air (France), **No. 54** (1934).

(47) SIEMENS, H. VON: Ann. Physik [4] **42,** 871 (1913).

(48) SMITH, A., AND MENZIES, A. W. C.: J. Am. Chem. Soc. **32,** 1448–59 (1910).

(49) SMITH, E. R.: J. Research Natl. Bur. Standards **24**, 229–34 (1940).
(50) SMITH, E. R.: J. Research Natl. Bur. Standards **26**, 129–34 (1941).
(51) SMITH, E. R., AND MATHESON, H.: J. Research Natl. Bur. Standards **20**, 641–50 (1938).
(52) SWIETOSLAWSKI, W., AND SMITH, E. R.: J. Research Natl. Bur. Standards **20**, 552 (1938).
(53) VOLMER, F.: Physik Z. **30**, 590–6 (1929).
(54) WARTENBERG, H. VON, AND BOSSE, O.: Z. Elektrochem. **28**, 384 (1922).
(54a) WILLINGHAM, C. B., TAYLOR, W. J., PIGNOCCO, J. M., AND ROSSINI, F. D.: J. Research Natl. Bur. Standards **35**, 219–44 (1945).
(55) WORINGER: Z. physik. Chem. **34**, 262 (1900).
(56) YOUNG, S.: J. Chem. Soc. **55**, 486 (1889).
(57) YOUNG, S.: Sci. Proc. Roy. Dublin Soc. **12**, 374–443 (1910).
(58) YOUNG, S.: Proc. Roy. Irish Acad. **38**, 65–92 (1928).
(59) ZMACZYNSKI, M. A.: J. chim. phys. **27**, 503–17 (1930).

MAGNETISM AND CATALYSIS

P. W. SELWOOD

Department of Chemistry, Northwestern University, Evanston, Illinois

Received September 21, 1945

The possibility of relationships between catalytic activity on the one hand, and magnetic fields and magnetic properties on the other, has interested many workers. It cannot be denied that those chemical elements which show the most pronounced catalytic activity, namely, the transition group elements, are also the elements which show the most interesting magnetic properties. In at least one case, the ortho–para hydrogen conversion, there is a clear-cut relationship between magnetic moment and catalytic activity. This is not to say that all catalytic activity is due to magnetism. It may yet appear that catalysis and magnetism are different manifestations of some more fundamental atomic property. But for the present, the chief applications of magnetism to catalysis are in structural studies of catalytic solids. A review of such applications, and of certain related effects, is the purpose of this article. Susceptibility data are included for some substances of major catalytic interest.

I. INTRODUCTION

It will be assumed that the reader is familiar with the principles of magnetochemistry (16, 132, 186, 192, 207); nevertheless, a few definitions will be given in this section, and some experimental methods will be briefly described.

Most substances are very slightly repelled by a magnet. These substances are said to be diamagnetic. The degree of diamagnetism does not vary greatly in different substances and has not, as yet, proved particularly useful in structural chemistry, in spite of considerable effort in this direction. Diamagnetism is independent of field strength and of temperature. It is actually a universal property of matter, because all substances have at least an underlying diamagnetism for which correction must be made for accurate determination of the permanent moment which may be present.

Many substances are slightly attracted to a magnet. These are paramagnetic. The effect is found especially among the transition group elements, and is related to the presence of incompletely filled energy levels. The existence of paramagnetism implies an unpaired electron. Atoms, ions, and molecules which have an odd number of electrons are invariably paramagnetic. Paramagnetism is independent of field strength at all ordinarily attainable fields. Very frequently the paramagnetism varies inversely as the absolute temperature, although many exceptions to this are known.

A few substances are very powerfully attracted to a magnet. The effect may be 10^3 to 10^6 times as strong as paramagnetism or diamagnetism. Such substances are said to be ferromagnetic. Ferromagnetism occurs only in iron, cobalt, nickel, and in a few alloys and compounds. Ferromagnetism is dependent on field strength in a complicated way, as shown in figure 1. It is also dependent on temperature, in that above a certain temperature, for nickel about 358°C., the ferromagnetism drops to zero and the substance becomes paramagnetic. The temperature of this sharp magnetic transition is called the Curie point.

If a substance is placed in a magnetic field of intensity H, and the magnetic induction in the substance is B, then

$$B = H + 4\pi\vartheta$$

where ϑ is the intensity of magnetization. The quantity

$$\vartheta/H = \kappa$$

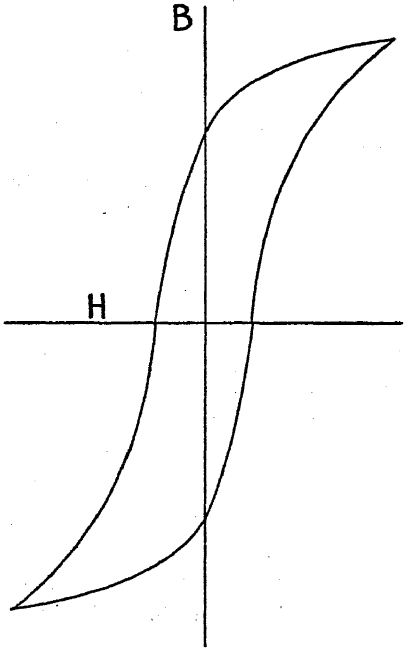


FIG. 1. Hysteresis curve showing dependence of induced magnetism on field strength for a ferromagnetic substance

is called the magnetic susceptibility per unit volume, and

$$\kappa/d = \chi$$

where d is the density, is the magnetic susceptibility per unit mass. This is the quantity with which magnetochemistry is chiefly concerned.

For paramagnetic substances χ often follows the Curie law

$$\chi = C/T$$

where C is the Curie constant and T the absolute temperature. More frequently χ follows the Weiss law

$$\chi = C/(T + \Delta)$$

where Δ is a constant. Deviations from both laws are common.

Atomic and molecular paramagnetism arises from the spin and orbital motions of the unpaired electrons. The orbital contributions are important only for the elements of the rare earth group. The effective magnetic moment, μ_{eff}, may be calculated from the susceptibility by the relationship

$$\mu_{\text{eff}} = \sqrt{\frac{3k\chi_m T}{N\beta^2}} = 2.83\sqrt{\chi_m T}$$

where k is the Boltzmann constant, χ_m is the molar susceptibility, T is the absolute temperature, N is Avogadro's number, and β is the Bohr magneton,

$$\frac{eh}{4\pi mc} = 0.917 \times 10^{-20} \text{ erg oersted}^{-1}$$

where e is the electronic charge, h is Planck's constant, m is the electronic mass, and c is the velocity of light. For precise determinations the molar susceptibility should be corrected for the underlying diamagnetism. But, owing to a variety of effects, μ_{eff} is seldom constant over a wide temperature range. In some cases use of the Weiss law gives somewhat better results, so that the expression for μ_{eff} becomes

$$\mu_{\text{eff}} = 2.83\sqrt{\chi_m (T + \Delta)}$$

Where the orbital contribution to the moment is negligible, the magnetic moment may be used to find the number of unpaired electron spins in the atom or molecule. The expression is

$$\mu_{\text{eff}} = \sqrt{n(n + 2)}$$

where n is the number of unpaired electron spins. The moment to be expected from one to five unpaired electron spins is as follows:

Unpaired electron spins	1	2	3	4	5
μ_{eff}	1.73	2.83	3.87	4.90	5.92

In a large number of cases the experimentally found magnetic moments agree with these values and, in general, deviations may be related to covalent binding and other types of interactions between atoms.

There are two principal methods for measuring the magnetic susceptibilities of solids. These are the methods of Gouy and of Faraday. The Gouy method, shown diagrammatically in figure 2, involves suspension of an elongated sample so that one end is in a strong magnetic field and the other in a region of negligible field intensity. The sample may be suspended from a sensitive balance. On application of the field the sample will appear to gain or lose in weight depending on whether the substance is paramagnetic or diamagnetic. The susceptibility is related to the force acting on the sample as follows:

$$f = 1/2\kappa H^2 A$$

where κ is the susceptibility per unit volume, H is the field strength, and A is the cross-section area of the sample. The apparatus is conveniently calibrated with water, for which $\chi = -0.720 \times 10^{-6}$, or with ferrous ammonium sulfate, Mohr's salt, for which $\chi = 9500 \times 10^{-6}/(T + 1)$, where T is the absolute temperature. The sample must be surrounded by a gas of negligible magnetic susceptibility. The susceptibility of air is in general not negligible, because oxygen is strongly paramagnetic. Nitrogen and hydrogen are convenient gases to use for surrounding the tube. The sample, if powdered, may be packed into a cylindrical glass tube. The uniformity of packing will generally limit the accuracy of the measurements. The fields used may be from 1000 to 15,000 or 20,000 oersteds. In practically all measurements on solids it is necessary to investigate the susceptibility at more than one field strength. This is because most solids contain minute traces of iron or other ferromagnetic impurities.

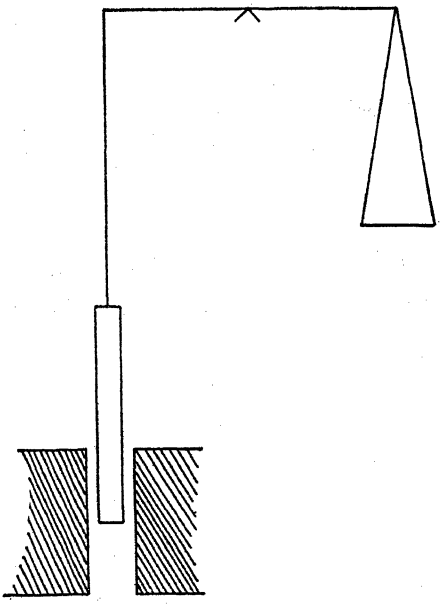


FIG. 2. Gouy magnetic balance

Such impurities lead to marked changes in susceptibilities. Correction for ferromagnetic impurities may be made by making the measurements above the Curie point of the impurities or, more simply, by plotting the susceptibility against reciprocal field strength. Extrapolation to infinite field gives substantially the susceptibility of the sample free from ferromagnetic impurities.

Measurements over a range of temperature may be made by surrounding the sample with an appropriately shaped Dewar flask into which are placed cooling liquids such as liquid air. High temperatures may be obtained by enclosing the sample in a long narrow heating coil. It is rather difficult to perform both high- and low-temperature experiments on the same apparatus.

In the Faraday method the sample may be much smaller. It is placed in a magnetic field which has a fairly sharp gradient along an axis in which the sample is free to move (figure 3). The force acting on the sample is given by

$$f = m\chi H \frac{dH}{dx}$$

where m is the mass of the sample, χ is the susceptibility per unit mass, H is the field strength, and dH/dx is the field gradient. The method is convenient and sensitive, small samples only are required, and no separate determination of the density is necessarily obtained. The sample may be suspended on a torsion arm, although a very large number of modifications have been suggested, using both horizontal and vertical displacement. For powdered solids the Faraday method appears to have some definite advantages over the Gouy method.

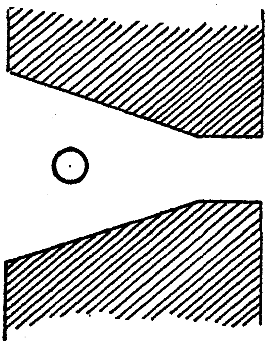


FIG. 3. Principle of the Faraday magnetic balance

Both the Gouy and the Faraday methods involve a motion of the sample. For the study of catalytically active substances it would be very convenient if the magnetic susceptibility could be determined *in situ*. For instance, it would be particularly useful if the preparation of the sample, measurement of catalytic activity, measurement of specific surface, and measurement of magnetic susceptibility could all be done on the same sample, without moving or transferring it in any way. There is, as yet, no method for doing this, but the development of such a method does not seem impossible. There are at least three approaches which would seem to offer promise. These are the inductance method, the Tobusch magnetometer, and the Rankine balance. They will be described briefly.

The inductance method—that is, the change in induction of a coil when a magnetic substance is placed in the coil—is, of course, a standard method for measuring the magnetic properties of ferromagnetic material such as steel. By use of a bridge and a sensitive galvanometer this method may be developed to measure susceptibilities of the order of 10^{-3}. A bridge for this purpose has been described by Elmore (42) after a design by Bitter. A diagram of the bridge is shown in figure 4. The sensitivity of this bridge would have to be increased about one thousand fold to make it suitable for susceptibility measurements on non-ferromagnetic samples. Very great sensitivity may be achieved by use of a heterodyne beat system, but efforts so far to adapt this to susceptibility measurements have failed. The problem does not, however, appear to be insurmountable. The measurement of samples *in situ* within a coil, without necessity for removing the sample or adjusting the container, would appear to be a little easier than measurements in which the sample would have to be removed. It should be pointed out that inductance methods are commonly used for the measurement of magnetic susceptibilities at extremely low temperatures.

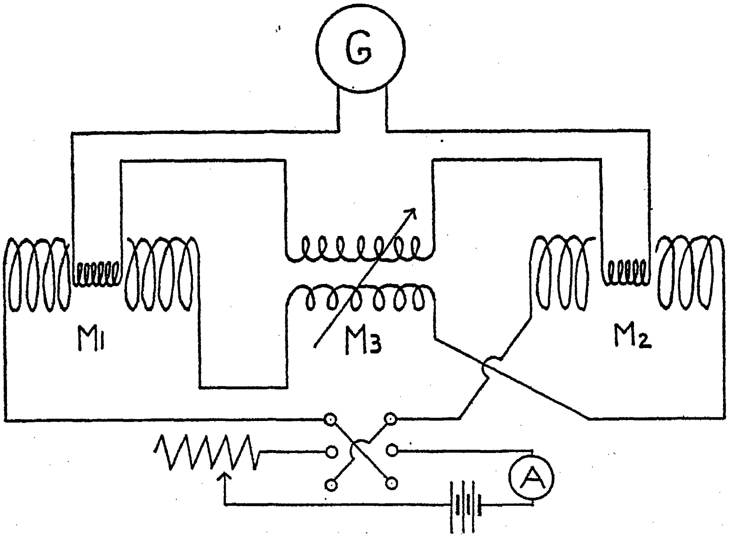


FIG. 4. Inductance bridge (Bitter–Elmore). G is a sensitive ballistic galvanometer; M_1 and M_2 are solenoids into one of which the sample is put. M_3 is a variable mutual inductance.

Another possible method for the *in situ* measurements of magnetic susceptibilities is the Tobusch magnetometer (see figure 5), as modified by Bozorth (29) and others (56, 177). The sample is placed inside one of two coils which are arranged on each side of an astatic magnet suspension system. The astatic system consists of two small rod-shaped permanent magnets arranged in opposition and placed a few centimeters apart on a rod attached to a delicate suspension fiber. Passing a current through the two solenoids has no effect on the suspended system unless one solenoid contains a substance of different susceptibility from the other. Movement of the system may be observed by a mirror and scale, and the system may be returned to a zero position by the use of a subsidiary coil placed inside one of the solenoids. The problem of increasing the sensitivity of this apparatus several hundred or thousand fold might include the use of Alnico or other alloys for the permanent magnets, and elaborate magnetic shielding.

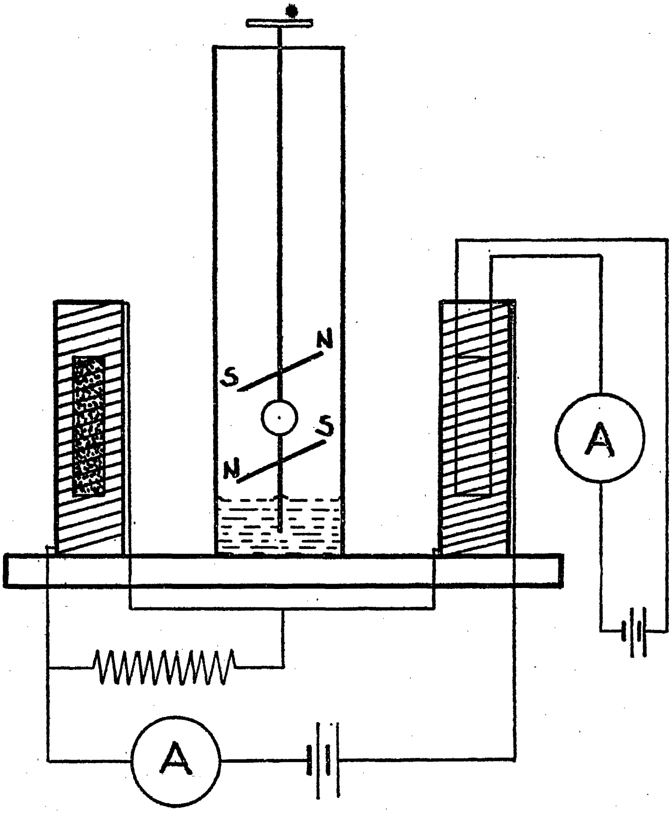


FIG. 5. Tobusch magnetometer

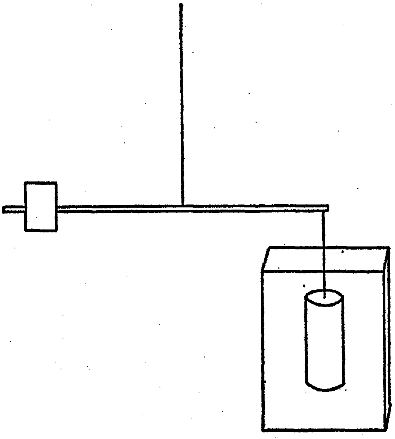


FIG. 6. Principle of the Rankine magnetic balance

The third possible *in situ* method is the Rankine balance (116, 174). In this method a small permanent magnet is suspended by a quartz-fiber system (figure 6) in such a way that the effect of stray fields is reduced. When a substance is placed near the permanent magnet a motion is induced in the suspended system. This method is sufficiently sensitive for measurements on catalytically active solids, but considerable difficulty is experienced in getting reproducible results. The field, all of which is produced by the permanent magnet, is quite small and may not exceed 100 oersteds.

Of course, it will be noted that in both the Gouy and the Faraday methods the desired result could be achieved if the magnet were made to move while the sample remained clamped in position. This is, however, a little difficult, because the sample may weigh a gram and the magnet half a ton. With the development of super-powerful permanent-magnet alloys one difficulty may be eliminated, but the effect of stray fields may continue to be a problem.

II. INFLUENCE OF A MAGNETIC FIELD ON CHEMICAL EQUILIBRIUM AND REACTION VELOCITY

The possibility that a magnetic field might have an effect on chemical reactivity has received investigation for well over one hundred years. It must be admitted that, although some interesting and curious effects have been discovered, none of these is very startling, and none has so far contributed very much to our understanding of either magnetism or catalysis.

The literature up to 1847 has been reviewed by Wartmann (211), who investigated the electrolysis of water and the electrodeposition of copper in a magnetic field. He found that the field had no detectible effects, in disagreement with a considerable number of workers who preceded him. In 1881 Remsen (178) discovered some beautiful effects when copper is displaced by iron in a magnetic field. The iron in the form of a shallow dish was placed above a magnet, and then copper sulfate solution was poured into the dish. "When copper is deposited from a solution of copper sulfate on a plate of iron in a magnetic field, it is arranged in ridges around the poles of the magnet, and in directions which are at right angles to the lines of force, and consequently, coincident with the lines marking the equipotential surfaces. Further the outlines of the poles of the magnet are always sharply defined on the plate, as along these lines a portion of the iron of greater or less width is left unacted upon."

Further work along these lines, together with various explanations of the phenomena, have been published by Rowland (180), Nichols (163), Loeb (143), Wolff (218), and Rathert (176). Loeb found no change in the velocity of the following reactions in a magnetic field of several thousand oersteds.

$$6FeSO_4 + KClO_3 + 3H_2SO_4 \rightarrow 3Fe_2(SO_4)_3 + KCl + 3H_2O$$

$$2HI + 2FeCl_3 \rightarrow I_2 + 2FeCl_2 + 2HCl$$

Wolff observed, incidentally, rotations of the solution during displacement reactions in a magnetic field. This seems to have been one of the first observations of the phenomenon recently reported as possibly being due to "magnetolytic" action. In the writer's opinion it is due to diffusion of ions of greater or less degree of hydration in the field.

Further studies of reactions between solutions and metals have been made by Berndt (10), Shchukarev (189), Rosenberg and Yuza (179), Forestier (48), and Parker and Armes (165). Some of these authors report an increase in reaction velocity, some a decrease, and some no change at all. It should be pointed out that the stirring effect automatically produced in such solutions in a field may serve to accelerate the reaction. This possibility is made more probable by the observation of Parker and Armes that vigorous mechanical stirring tends to reduce the difference in reaction velocity between the mixtures in and out of the field.

An isolated observation by Jahr (117) is to the effect that a photographic plate may be affected by the presence of magnetized iron.

As for homogeneous reactions, negative results for the application of a magnetic field have been found by de Hemptinne (36) for the hydrolysis of methyl acetate and the inversion of sucrose, by Garner and Jackman (52) for the mutarotation of dextrose and the hydrolysis of sucrose both with and without the addition of paramagnetic salts, by Henglein (80) for the velocity of the reaction

$$2NO + Cl_2 \rightleftharpoons 2NOCl$$

and by Kornfeld (140) for the reaction

$$2NO_2 \rightleftharpoons O_2 + 2NO$$

Clark and Archibald (31) found that the reaction products of nitric acid on benzoic acid did not vary appreciably whether in a field or out.

Rates of crystallization in a magnetic field have been studied fairly recently by Steacie and Stevens (191) and by Berlaga and Gorskiĭ (9). No appreciable change in rate was found for such diverse substances as sodium thiosulfate, nickel sulfate, water, salol, and diphenylamine.

Jennison (118) has reported no change in the growth and other properties of bacteria, yeast, and molds on exposure to fields of 3000 oersteds.

In spite of this array of negative results, there are theoretical reasons for believing that a magnetic field might affect both reaction velocity and equilibrium, for certain types of chemical reactions (124).

Bhatnagar, Mathur, and Kapur (15) have studied a fairly large number of reactions in fields of from 600 to 1700 oersteds. They point out that in non-uniform fields the paramagnetic constituents of reaction mixtures may suffer local changes in concentration, and that in uniform, as well as non-uniform, fields the orientation of the elementary magnetic moments may affect the effective collision frequency. The theory of these effects may require further elaboration, and the experimental results are complicated by the difficulty of separating what might be called the magnetochemical phenomena from the purely mechanical. Nevertheless, the results reported by the Indian workers are illuminating. In brief, for those reactions in which the algebraic sum of molar susceptibilities increases, the rate of reaction is accelerated by the field. For those in which the susceptibility sum decreases, the reaction is retarded by the field. That this rule is fairly general is indicated by the following series of reactions.

	$\Sigma\chi_{M_i}$	$\Sigma\chi_{M_f}$
A. Reactions accelerated by the magnetic field:		
1. Zinc plus dilute hydrochloric acid	149.6	44624.96
2. Permanganate plus oxalic acid		
3. Ferric chloride plus iron		
4. Ferric chloride plus aluminum		
5. Chromic acid plus phosphorous acid	−47.72	3774.48
6. Chromic acid plus organic acids		
7. Chromic acid plus ammonium oxalate		
8. Chromic acid plus potassium iodide	−336.48	3499.7
9. Potassium permanganate plus chloral hydrate	329.48	4658.8
B. Reactions retarded by the magnetic field:		
1. Ferric chloride plus zinc		
2. Iron plus dilute hydrochloric acid		
3. Ferric chloride plus hydroxylamine		
C. Reactions not affected by the magnetic field:		
1. Aluminum plus dilute hydrochloric acid		
2. Ferrous sulfate plus iodide plus iodate		
3. Cobaltous sulfate plus iodide plus iodate		
4. Hydriodic acid plus hydrogen peroxide	−111.66	−114.42
5. Acetic acid plus alcoholic hydrogen chloride	−65.08	−65.1
6. Mononitration of phenol		

In most cases the change in velocity constant, when a change was observed, was of the order of 1 per cent. For some of the homogeneous reactions there are given the initial ($\Sigma\chi_{M_i}$) and the final ($\Sigma\chi_{M_f}$) total susceptibilities. For these cases, at any rate, it seems to be true that when $\Sigma\chi_{M_f} > \Sigma\chi_{M_i}$ the reaction is accelerated, and *vice versa*.

The patent literature contains occasional references to control of reaction velocity by magnetic fields of greater or less intensity. Several of these have been investigated by the writer, all with uniformly negative results.

III. THE ORTHO–PARA HYDROGEN CONVERSION

Catalysis of the ortho–para hydrogen conversion by paramagnetic substances is a well-known phenomenon, adequately described by Farkas (44) and by Cremer (35). This conversion has been treated theoretically by Wigner (216) and by Kalckar and Teller (123). The homogeneous reaction is catalyzed by the paramagnetic gases, oxygen, nitric oxide, and nitrogen dioxide, and by solutions containing paramagnetic ions such as Cu^{++}, Ni^{++}, Co^{++}, Fe^{++}, Mn^{++}, and rare earth ions. Aqueous solutions of oxygen are also active. The heterogeneous reaction is catalyzed by paramagnetic solids such as Cr_2O_3, Gd_2O_3, Nd_2O_3, and V_2O_3, while it occurs at a much lower velocity over weakly paramagnetic or diamagnetic solids such as CeO_2, ZnO, and La_2O_3 (195, 196). The low-temperature heterogeneous reaction is also strongly catalyzed by charcoal, which is diamagnetic. The conversion may be due in this case to the so-called "surface paramagnetism" which has, in one or two instances, apparently been observed experimentally (57, 96).

In general, those paramagnetic solids showing catalytic activity also exhibit strong hydrogen adsorption at low temperatures. This is, however, not invariably true, as is shown by the work of Turkevich and Selwood (204). The organic free radical α,α-diphenyl-β-picrylhydrazyl is strongly paramagnetic, but it does not catalyze the ortho–para hydrogen conversion. This seems to be because the free radical, which is a stable solid, does not appreciably adsorb hydrogen. Zinc oxide, on the other hand, strongly adsorbs hydrogen but exhibits only a weak catalytic effect. If these two substances, the free radical and the zinc oxide, are thoroughly ground together, the mixture acquires strong catalytic activity (figure 7). There are apparently two factors necessary for the heterogeneous low-temperature catalysis: namely, an inhomogeneous field, produced in this case by the free radical, and adequate contact of the hydrogen with the field, ensured by van der Waals adsorption of the hydrogen on the zinc oxide.

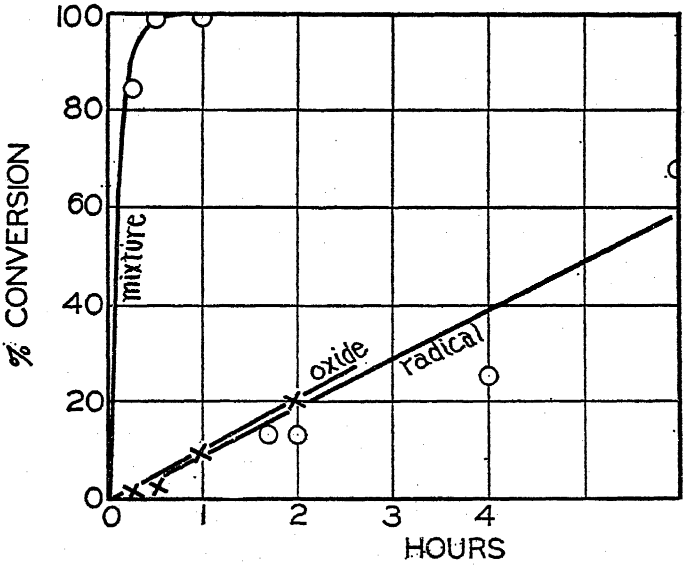


Fig. 7. Ortho–para hydrogen conversion on zinc oxide, on a free radical, and on a mixture of the two

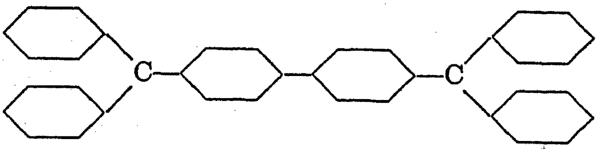
Homogeneous magnetic fields, incidentally, do not promote the ortho–para hydrogen conversion. Some diamagnetic substances containing hydrogen, such as water, slowly catalyze the reaction, presumably because of the inhomogeneous field due to the nuclear magnetic moment of the hydrogen.

There have been a large number of applications of paramagnetic catalysis of ortho–para hydrogen conversion. Unfortunately, these have not proved quite so important as was at one time hoped. This is because, unlike the great majority of chemical reactions, no valence bond is ruptured in the low-temperature paramagnetic reaction. In recent years the principal use of the ortho–para conversion has been in verifying the presence of free radicals or other paramagnetic substances in systems which do not lend themselves readily to the usual methods of susceptibility measurement. A few instances of such applications will be given.

The structure of diborane, B_2H_6, has excited discussion for some time because there are insufficient electrons in this molecule to provide electron-pair bonds throughout. The existence of unpaired electrons, or single-electron bonds, would presumably give rise to paramagnetism. Diborane is scarcely stable enough for the usual magnetic susceptibility methods; Farkas and Sachsse (45) have found that this substance does not appreciably catalyze the ortho–para hydrogen conversion. The molecule cannot, therefore, be paramagnetic. This conclusion seems to have been verified by direct susceptibility measurements (51).

Similarly, a study has been made by Eley (41) on the heterogeneous conversion on a variety of porphyrin compounds, including hematoporphyrin, hematin, hemin, metal-free phthalocyanine, and copper phthalocyanine. As expected, the conversion is catalyzed by the paramagnetic but not by the diamagnetic solids.

In some cases the ortho–para hydrogen method yields results which could not have been obtained by the direct measurement of susceptibility, even though the experimental difficulties in the latter should be overcome. Schwab (185) has found that the Chichibabin hydrocarbon



catalyzes the ortho–para reaction to a degree indicating about 9.7 per cent dissociation to the biradical, although Müller, by direct measurement of susceptibility, has shown this compound to be diamagnetic.

The effects described for the ortho–para hydrogen conversion have suggested to several workers that local non-uniform magnetic fields in the neighborhood of paramagnetic atoms or ions might be a more general cause of catalytic action than has hitherto been suspected. This possibility has been advanced by Ogawa (167), Kitagawa (125), and Kuhn (141). Experiments by Gilbert, Turkevich, and Wallis (53) have not, however, substantiated this idea. No correlation was found between susceptibility and catalytic activity in the *cis–trans* isomerization of dimethyl maleate. The catalysts used, the magnetic susceptibilities, and the time necessary to form crystals of dimethyl fumarate from the maleate are shown in table 1. In addition to these substances, nickelous chloride, magnesium chloride, mercuric chloride, mercurous chloride, water, and Fe_2O_3 gave no crystals in 5 hr.

IV. POLYMERIZATION REACTIONS

There have been a few studies on magnetic changes during polymerization (5, page 61) but, so far as the writer is aware, only one short paper has appeared on the magnetic detection of free radicals in a polymerizing system. Farquharson and Ady (43) report that 2,3-dimethylbutadiene undergoes, in the absence of promoter, a 13.6 per cent diminution of diamagnetism, followed by a normal rise. If this observation can be definitely related to the presence of free radicals it promises to be of considerable interest in the study of polymerization kinetics. The methods used at present for susceptibility measurements may have to be refined considerably before accurate estimates of free-radical concentration in such systems may be made. But there is no particular reason why this cannot be done, because all that is required is the relative susceptibility before, during, and after polymerization. Such measurements, of the type already used by Michaelis in the study of semiquinone ions (186, page 130), may be made with considerably more accuracy than may those in which the sample has to be removed between determinations.

TABLE 1

Conversion of dimethyl maleate to dimethyl fumarate

CATALYST	$\chi \times 10^6$	TIME
Na	0.51	10 min.
$AlCl_3$	−0.60	60 min.
$FeCl_3$	86.0	180 min.
$ZnCl_2$	−0.47	300 min.
$CrCl_3$	44.0	9 hr.
Fe_3O_4	Ferromagnetic	15 hr.

A problem closely related to the above, and one that merits magnetochemical study, is the dissociation of diaryl peroxides. No magnetic measurements appear to have been made on aroxy radicals, although Bachmann (4) describes several, such as the 9-methoxy-10-phenanthroxy radical, which are supposed to have a considerable degree of stability. The relation to polymerization kinetics is, of course, through the use of diaryl peroxides as accelerators. There is no *a priori* reason why even benzoyl peroxide should not be slightly dissociated, and it is rather surprising that no magnetic studies have been published on this compound.

V. MAGNETIC STATE AND CATALYTIC ACTIVITY

Hedvall (32, 33, 69–79) and his coworkers have extensively investigated the catalytic activity of ferromagnetic substances in the neighborhood of the Curie point. Work has been published on the decomposition of nitrous oxide, the hydrogenation and the decomposition of carbon monoxide, the hydrogenation of ethylene, of castor oil, and of cottonseed oil, and the cleavage of formic acid. These reactions have been studied on a variety of ferromagnetic solids, with a variety of Curie temperatures. The results, in brief, show that at the Curie point there is a sharp increase in the temperature coefficient of reaction velocity. The results will be described more fully in connection with the decomposition of nitrous oxide and the disproportionation of carbon monoxide.

Nitrous oxide was passed over pure nickel, the Curie temperature of which was 358–361°C. The velocity of the decomposition reaction $2N_2O \rightarrow 2N_2 + O_2$ was measured by the increase of volume, and later by a continuous gas analyzer. As shown in figure 8, there is a sharp increase in the temperature coefficient of reaction velocity at the Curie temperature. This is not an accidental coincidence, because various alloys of nickel, with lower Curie points, show similar changes in reaction velocity. Copper–nickel alloy, for instance, with a Curie temperature of 337–340°C., shows a sharp change in the temperature coefficient of reaction velocity at almost exactly 339°C.

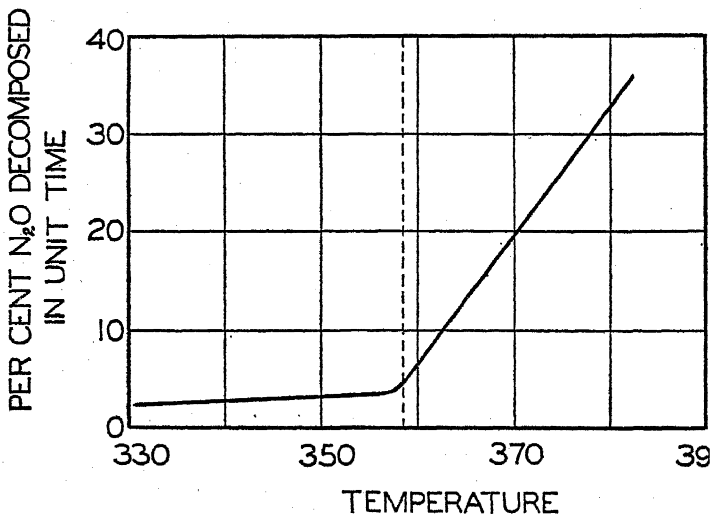


Fig. 8. Catalytic activity of nickel near the Curie point

With the reaction $2CO \rightarrow C + CO_2$ the results are similar. It should be pointed out that these striking results are obtained only when the Curie temperature is well defined. Ordinary commercial nickel shows a rather gradual decrease of magnetization with increasing temperature, the change occurring over 50° or 100°. Such nickel shows only a correspondingly gradual change in catalytic activity. But highly purified nickel shows a sharp Curie point, the total drop in magnetization occurring within 3° or 4°. It is with such substances that Hedvall's most interesting results occur. A rather large group of ferromagnetic catalysts has been studied in this way. These include, besides nickel and nickel alloys, magnesium ferrite, cobalt–palladium alloy, pig iron, tungsten–chromium steel, and Heusler alloys. The sudden application of a magnetic field does not affect the reaction rate, either above or below the Curie point.

These changes, sometimes called the "Hedvall effect", have been studied by several other investigators, not all of whom have been able to obtain similar results. Fischbeck (46) found a change in the synthesis of ammonia on γ- and α-iron, respectively almost non-magnetic and strongly ferromagnetic. The ferromagnetic type of ferric oxide is also a better catalyst than the other type for the decomposition of potassium chlorate (13). But Fischbeck (47) was unable to confirm Hedvall's observation on the reaction of carbon dioxide with hydrogen over nickel or nickel alloys.

The existence of some kind of change in catalytic activity at the Curie point has been confirmed by Aoyama (2) for the hydrogenation of ethylene over copper–nickel alloy and by Forestier and Lille (49) for the decomposition of acetic acid over a variety of ferrites. But the changes take the form of an acceleration of catalytic activity at the Curie point, followed by a decrease of activity above the Curie point. The effect reported is, therefore, somewhat different from the Hedvall effect. Related effects are reported by Veil (208).

Olmer (168, 169) was unable to observe the Hedvall effect for the catalytic decomposition of carbon monoxide over iron, cobalt, nickel, and the compounds CMn_3, BMn, and SbMn, all of which are ferromagnetic. It is not clear why these experiments gave negative results, unless the Curie temperatures of the particular samples used were too indefinite to show the sharp changes in catalytic activity to be expected.

Körber, Wiemer, and Fischer (139) find that carbon monoxide undergoes a more rapid decomposition at about 900°C. over pure iron, but they attribute this less to catalytic activity than to reaction between the iron and the carbon monoxide. They show, by appropriate magnetic measurements, that powdered iron in contact with carbon monoxide above 500°C. is transformed entirely into cementite. Further extended criticism of the Hedvall effect has been given by Spingler and Reinhard (190). In experiments closely paralleling those of Hedvall they observed no discontinuity for the decomposition of carbon monoxide over nickel. In the hydrogenation of carbon monoxide there is an increase in activity beginning at about 350°C. and ending with a sudden decrease at about 370°C. These changes are, however, apparently independent of the Curie point. These authors believe that the decomposition of carbon on the catalyst may account for the observed changes in the neighborhood of the Curie point of nickel. These criticisms quite obviously do not explain the Hedvall experiments in which the catalytic discontinuity was found at lower temperatures when the Curie point of nickel was lowered by the use of copper–nickel alloys.

In spite of these criticisms it seems difficult not to believe in the reality of the Hedvall effect. But in the absence of any theoretical explanation for this effect we shall devote no further space to it.

Various other miscellaneous relationships between magnetic state and catalytic activity have been noted by Veil (209), by Baudisch (6, 7), and by Scheepers (183).

VI. PRINCIPLES OF STRUCTURAL MAGNETISM

The principal problem of magnetochemistry as applied to catalysis is chiefly a problem in structural inorganic chemistry. It is to gather information about the identity, valence relationships, and atomic environment of the active substance. In this sense magnetic measurements have proved to be unusually revealing, and promise to be even more so, with respect to the structure of catalytically active solids. In this section some general principles will be given, to be followed by detailed remarks on a large variety of substances of interest to specialists in the field of catalysis.

A substance may, like aluminum oxide, be diamagnetic according to theory, yet slightly paramagnetic by observation. This is a common occurrence and almost always reveals the presence of some paramagnetic impurity in the substance. Paramagnetism generally varies inversely as the absolute temperature. Hence the presence of paramagnetic impurities may often be made much more conspicuous by making the measurements at low temperatures. In this way a very small fraction of 1 per cent of ferric oxide, present as impurity, could easily be detected in alumina.

TABLE 2

Valence states and effective magnetic moments in the first transition series

Unpaired electrons......	0	1	2	3	4	5
Calculated moment.....	0	1.73	2.83	3.87	4.90	5.92
Observed moment.......	0	1.77–1.79	2.76–2.85	3.7–4.0	4.8–5.0	5.2–6.0
Ions....................	Sc^{+3} Ti^{+4} V^{+5} Cu^{+1} Zn^{+2}	Ti^{+3} V^{+4} Cu^{+2}	Ti^{+2} V^{+3} Ni^{+2}	V^{+2} Cr^{+3} Mn^{+4} Co^{+2}	Cr^{+2} Mn^{+3} Fe^{+2} Co^{+3}	Mn^{+2} Fe^{+3}

There are some substances of catalytic interest which show a slight paramagnetism which is independent of temperature. Such a substance is ceric oxide. But this temperature-independent paramagnetism is easily distinguished from a normal paramagnetic impurity by making measurements at two or more temperatures.

If a substance is normally paramagnetic, the magnetic moment in Bohr magnetons may be calculated from the expression

$$\mu_{eff} = 2.83 \sqrt{\frac{3k\chi_m (T + \Delta)}{N\beta^2}}$$

as previously given. For nearly all transition group elements except the rare earths, the moment is related to the number of unpaired electron spins. The number of unpaired spins is, of course, related to the valence. The number of unpaired electrons, the calculated, and the average observed magnetic moments for several elements in the first transition series are given in table 2.

The magnetic susceptibility often gives, therefore, a means of finding the valence state. In mixtures of solids, because the susceptibilities are, to a first approximation, additive, the valence states may sometimes be found magnetically when other methods cannot be applied.

The data given in table 2 apply generally to substances which are magnetically dilute. By this is meant that the paramagnetic ions are situated relatively far apart in the crystal. Increasing interaction between paramagnetic atoms or ions is indicated by increasing magnitude of the quantity Δ in the Weiss law. This quantity may definitely be related to the degree of interaction between paramagnetic ions. For instance, chromic oxide (Cr_2O_3) has a strong paramagnetism almost independent of temperature. But dilute solid solutions containing the Cr^{+3} ion have a normal temperature coefficient. The quantity Δ is therefore useful in following the dispersal, or aggregation, of paramagnetic ions in solids, especially in diffusion processes. It often happens that compounds expected to be paramagnetic, such as molybdenum sulfide, turn out to be diamagnetic, or to have a greatly reduced paramagnetism. This is generally due to interaction between the paramagnetic ions and to covalent-bond formation in the compound. The mechanism for this change will be described below.

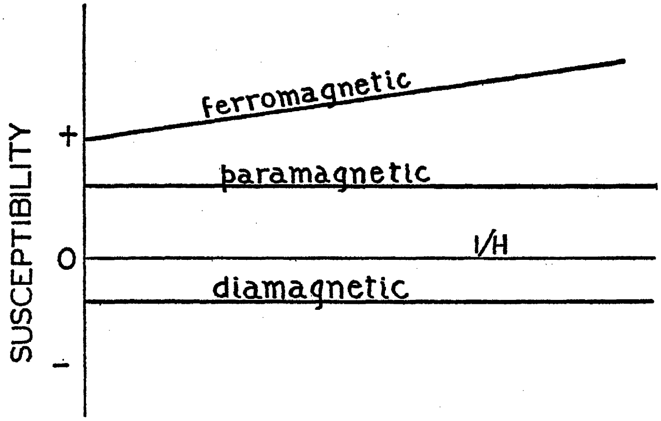


FIG. 9. Plot of reciprocal field strength against susceptibility for diamagnetic, paramagnetic, and ferromagnetic substances.

Almost every solid has a trace of ferromagnetic impurity. This may be due to magnetic oxide of iron (Fe_3O_4) derived from dust or to other causes. Solids prepared in contact with steel containers at any step are generally rich in ferromagnetic content. For most carefully prepared solids the ferromagnetism reveals itself as a slight field strength dependence of susceptibility. This is neatly shown by plotting the susceptibility against reciprocal field strength, as shown in figure 9. Strictly pure diamagnetic and paramagnetic substances have susceptibilities independent of field strength. The magnitude of the ferromagnetism is expressed as the specific magnetization, σ, and is equal to the intensity of magnetization, ϑ, divided by the density. It is also given by χH, where χ is the susceptibility per gram and H is the field strength. It is not infrequently possible to estimate the quantity of ferromagnetic substance present provided its nature is known. Specific magnetizations are well known for most ferromagnetic substances and the proportion is roughly given by σ/σ_0, where σ is the observed magnetization and σ_0 that of the pure ferromagnetic substance. This method is unusually sensitive; often as little as 1 part in 10^9 of iron may be detected. Sometimes, if the impurity happened to be, say, paramagnetic Fe_2O_3, it could be made easily detectible by first heating the sample in hydrogen. In this way any "latent" ferromagnetism may be "developed" to a point where it is measurable. Measurements of ferromagnetism are especially useful in deciding whether one of the ferromagnetic elements is present as metal or in compound form.

The nature of a ferromagnetic constituent may often be found by observation of the Curie temperature. For pure substances the Curie temperature is often well defined, that for nickel being close to 358°C. For mixtures, such as alloys, the temperature–magnetization curve often reveals the nature, and distribution of concentrations, of the various substances present.

The application of magnetic measurements to complex compounds is due chiefly to Pauling (172). The method will be illustrated with respect to a nickel cyanide complex, although the chief application, so far as catalysis is concerned,

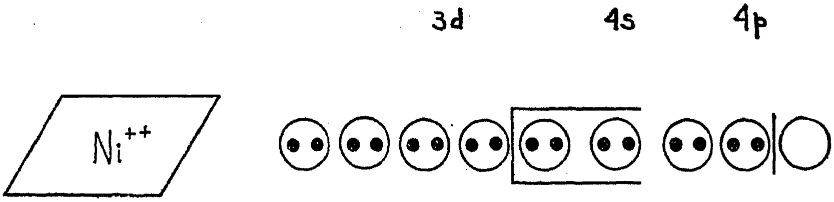


FIG. 10. Electron configuration in a planar covalent nickel complex. The free nickel ion has two unpaired 3*d* electrons.

has been in connection with natural complexes containing iron. Divalent nickel has two unpaired electrons in the 3*d* orbital, as shown in figure 10. The magnetic moment for magnetically dilute compounds containing this ion is normal,—namely, about 3 Bohr magnetons. In the cyanide complex, $K_2Ni(CN)_4$, however, the nickel becomes diamagnetic, presumably owing to pairing of all electrons. A way in which this might be done is to use the 4*s*, two of the 4*p*, and one of the 3*d* orbitals for the formation of covalent bonds. According to the quantum-mechanical interpretation of directed valence, dsp^2 bonds should be square and planar, while four ionic bonds would, of course, be tetrahedral. It is, therefore, concluded from the diamagnetism of potassium nickelocyanide that the cyanide groups lie in a plane. This view is confirmed by x-ray studies. This interpretation is most effective for complexes containing nickel, but is also applicable to other transition group elements.

There remains to mention the rare earth elements. Their observed magnetic moments correspond to a rather complicated interaction of spin and orbital moments. So far as magnetism and catalysis are concerned, the chief interest of the rare earths lies in valence state determinations, and, of course, in the ortho–para hydrogen conversion.

VII. STRUCTURAL STUDIES

Morris and Selwood (162) have made parallel magnetic and catalytic measurements on nickel-activated copper. The reaction chosen was the hydrogenation of benzene at 175°C. Catalysts containing 1 per cent of nickel in metallic copper were prepared by mixing nickel nitrate with precipitated copper hydroxide, drying, igniting, and reducing in hydrogen at a low temperature. The copper was specially purified for this work.

The results show that an active catalyst is quite strongly ferromagnetic, and that heating the catalyst produces a loss of ferromagnetism, as observed at room temperature, and that this loss of ferromagnetism occurs in linear relationship to the loss of catalytic activity (figure 11). The explanation for these changes is made clear by determinations of the Curie point before, during, and after thermal inactivation. Use is made of the facts that the Curie point of a homogeneous copper–nickel alloy is approximately a linear function of the concentration (144), and that the specific magnetization below the Curie point is also approximately linear with concentration (115). Figure 12 shows magnetization as a function of temperature for a copper catalyst activated with 2 per cent of nickel. It will be noted that at the temperature of the hydrogenation reaction, 175°C., the active catalyst is only a little ferromagnetic, and the partially deactivated catalyst is not ferromagnetic at all. Apparently it is by no means essential that the active mass should be ferromagnetic at the temperature of the reaction.

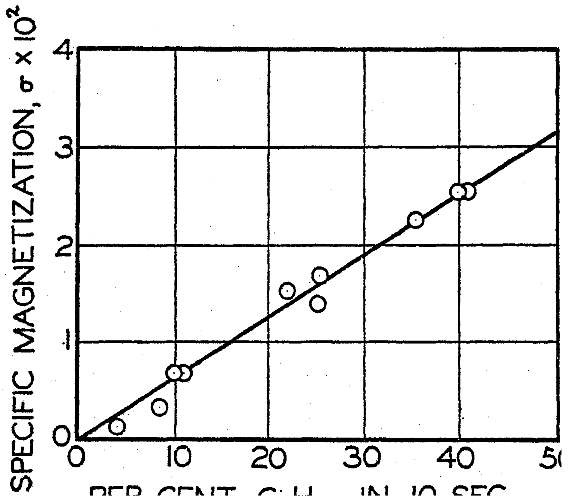


FIG. 11. Linear relationship between magnetization and catalytic activity

The active catalyst can, on the basis of these results, contain no unalloyed metallic nickel in aggregates greater than those containing about 10^3 atoms (67, 160). Smaller aggregates are not ferromagnetic. The highest concentration of nickel alloy present in the active catalyst seems to be about 92.5 per cent, but most of the nickel is present in a much lower concentration. It is clear also that most of the active mass is inhomogeneous, because the magnetization curve shows no abrupt change, but rather falls quite slowly as the temperature is raised. It seems probable, therefore, that the catalytic activity is due, not to metallic nickel as such, but to the nickel-rich nickel–copper alloys shown to be present. Figure 12 shows that thermal deactivation is attended by a diffusion process whereby the nickel gradually becomes uniformly distributed throughout the mass. The process is essentially a dilution by copper of the nickel-rich nickel–copper alloys present in the active mass. The quantitative treatment of this diffusion process in powdered metals is given by Selwood and Nash (187).

There are two observations to be made in connection with the above studies. The first is that no parallel study of specific surface was attempted. It will be difficult to relate magnetic properties to catalytic properties during thermal treatment until some way is found to control, or correct for, the surface changes which must obviously take place under such treatment. The second observation is that the published data show only a small proportion of the nickel to be accounted for as contributing to the ferromagnetism of the active catalyst. The remaining nickel must be present as unreduced nickel oxide, as atomically dispersed nickel on the surface or interstices of the copper crystals, or possibly as dissolved nickel in dilute solution. The state of this "non-ferromagnetic" nickel and its influence, if any, on catalytic activity are problems of considerable importance.

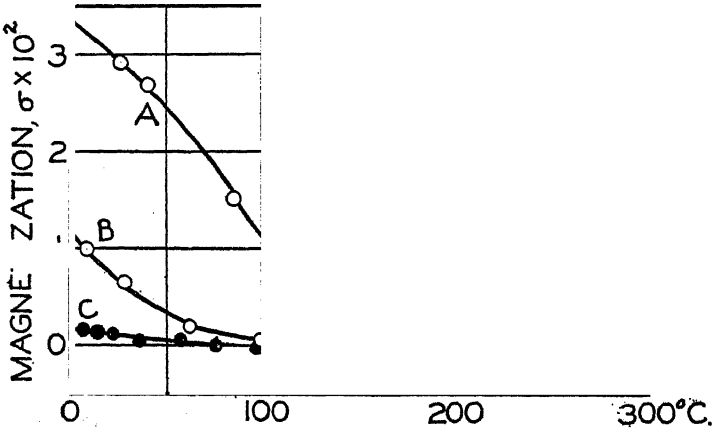


FIG. 12. Magnetization and Curie point of a nickel-activated copper catalyst. A, active catalyst; B, partially deactivated catalyst; C, inactive catalyst.

Further measurements by the same authors (71) have been made on nickel supported on magnesia. In this case, practically all the nickel is accounted for as contributing to the ferromagnetism. Thermal deactivation does not lead to a recession of the Curie point for this catalyst. The same is true of poisoned catalysts, in that only a slight change in magnetization occurs when a nickel-activated copper catalyst is treated with a variety of catalyst poisons.

The magnetocatalytic properties of molybdenum oxide derivatives have been studied by Woodman, Taylor, and Turkevich (219). Their most significant results are in connection with the catalyst obtained by heating nickel ammonium molybdate, first in air, then in hydrogen, at 300°C. This catalyst is active in the hydrogenation of ethylene at −80°C. The catalyst is strongly ferromagnetic, a property which the authors attribute to metallic nickel. But Morris and Selwood (71) made magnetic measurements on a similar catalyst over a range of temperature and found that the Curie interval was large, and that all ferromagnetism was lost at 250°C. Metallic nickel is not, therefore, proved to be present. But what the ferromagnetic component is, is not known. The total ferromagnetism at room temperature amounts to little over 10 per cent of that to be expected if all the nickel present were in the form of metal.

The above work is a good example of the use of magnetic studies in catalysis. It also illustrates the necessity of extending magnetic measurements over a considerable temperature range, including the regions of all pertinent critical points. It should be pointed out that nickel-containing catalysts prepared as above, even with rather vigorous heating in hydrogen, do not necessarily show ferromagnetism. What, precisely, are the conditions necessary for the development of ferromagnetism in such catalysts we are not prepared to say. But in every case which has come to the writer's attention the presence of ferromagnetism has been paralleled by some striking changes in catalytic activity.

Several attempts have been made by Bhatnagar and his coworkers to find the nature of transitory compounds and valence states formed during the course of reactions. The catalytic decomposition of potassium chlorate by manganese dioxide (21), ferric oxide (13, 21), and cobalt oxide (13) is one such reaction. Mixtures of potassium chlorate and of each catalyst in turn were heated to various temperatures for definite time intervals. The mixtures were then analyzed and tested for magnetic susceptibility. For the manganese and cobalt oxides the susceptibility shows a negative deviation from additivity amounting to about 5 per cent. The intermediate compound must be either diamagnetic or less strongly paramagnetic. Ferric oxide showed no deviation from additivity although, as previously mentioned, ferromagnetic γ-Fe_2O_3 proved to be a better catalyst than paramagnetic α-Fe_2O_3 (13). Evidence of intermediate compounds has also been obtained for the chlorination of chloroform to carbon tetrachloride in the presence of ferric chloride (24), for the catalysis of the reaction between ammonium oxalate and mercuric chloride by ferric ions (14), and for the catalysis of the persulfate–iodide reaction by ferric ions (22). A related type of study has been made by Merck and Wedekind (149, 150) on the oxidation of carbon monoxide as catalyzed by manganese dioxide, by cobaltic oxide, and by the mixed oxides and oxide hydrates.

The catalytic action of cupric ion on the oxidation of *l*-ascorbic acid has been known for some time. Tyson and Wiley (205) have used magnetic measurements to determine the function of the cupric ion in this reaction, as carried out in pyridine solution. Cupric ions in pyridine have a magnetic moment corresponding roughly to one unpaired electron spin. Mixed with an appropriate quantity of ascorbic acid, the copper becomes diamagnetic, presumably owing to the reaction:

$$2Cu^{++} + \text{ascorbic acid} \rightarrow 2Cu^{+} + 2H^{+} + \text{dehydroascorbic acid}$$

Exposure to oxygen or air results in changing the susceptibility back to a value corresponding to one unpaired electron spin for the copper. The colors of the solutions suggest that both cuprous and cupric ions form complexes with the dehydroascorbic acid.

A similar type of valence study by Noyes, Pitzer, and Dunn (164) shows, on the basis of magnetic and other measurements, that silver ions oxidized by ozone in concentrated nitric acid exist in the paramagnetic bipositive form.

Determination of bond type in enzymatic and related substances of biological interest is somewhat remote from the primary purpose of this review, but for the sake of completeness references will be given to work in this field. The extensive studies of Pauling and Coryell and their coworkers on hemoglobin and derivatives have been reviewed by Drabkin (37) and by the writer (186). Theorell has published studies on cytochrome (197), horseradish peroxidase (198), and horse-liver catalase (199). Catalase has also been investigated magnetically by Michaelis and Granick (156), who have further published an extensive study on ferritin (157).

VIII. ACTIVE OXIDES

In recent years there have been a large number of studies on mixed oxides. Most of this work is due to Hüttig (88–92) who, fortunately, has prepared reviews covering several hundred papers in this field. In general, the procedure has been to mix oxides, of which zinc oxide and ferric oxide will serve as examples, and to follow changes in catalytic activity, magnetic susceptibility, and other properties, as reaction in the solid state proceeds with the ultimate formation of a single compound. In the case given the final product would be a spinel, $ZnFe_2O_4$. In a large number of instances there are indications that intermediate compounds are formed, and these often have very pronounced catalytic and magnetic properties. The procedures used in the preparation of these compounds are often different from those commonly used in the preparation of active catalysts. For instance, Hüttig and his coworkers have studied copper chromite as formed by the reaction

$$CuO + Cr_2O_3 \rightarrow CuCr_2O_4$$

The reaction occurs at a high temperature, under conditions such that the final product is a poor catalyst, and is probably quite different from the well-known substance formed by thermal decomposition at moderate temperatures of copper ammonium chromate. Nevertheless, the substances studied by Hüttig are, in a large number of cases, of interest to catalytic chemists because his products are often formally related to some of the most important industrial and laboratory types of catalysts.

In the following discussion a few examples will be selected. References will be given to many other systems.

The system $CaO{-}Fe_2O_3$ (99, 109, 129, 130): If calcium oxide and ferric oxide are heated together, there begins to appear in the neighborhood of 800°C. unmistakable evidence for spinel formation. This change is clearly shown by x-ray diffraction, and is also indicated by the onset of ferromagnetism, of greatly reduced catalytic activity, and of numerous other changes in physical properties. Prior to this change, however, there are changes in susceptibility and a pronounced increase in catalytic activity. These changes, which are shown in figure 13, are paralleled by other changes. The experimental procedure consists in preparing stoichiometrical mixtures of the oxides, heating them for definite intervals (generally 6 hr.) at numerous gradually increasing temperatures, and, between heating periods, measuring the properties of the mixture under standardized conditions. Thus the magnetic susceptibility is generally found at 21°C. at several field strengths. The catalytic activity has been determined for the oxidation of carbon dioxide and the decomposition of nitrous oxide.

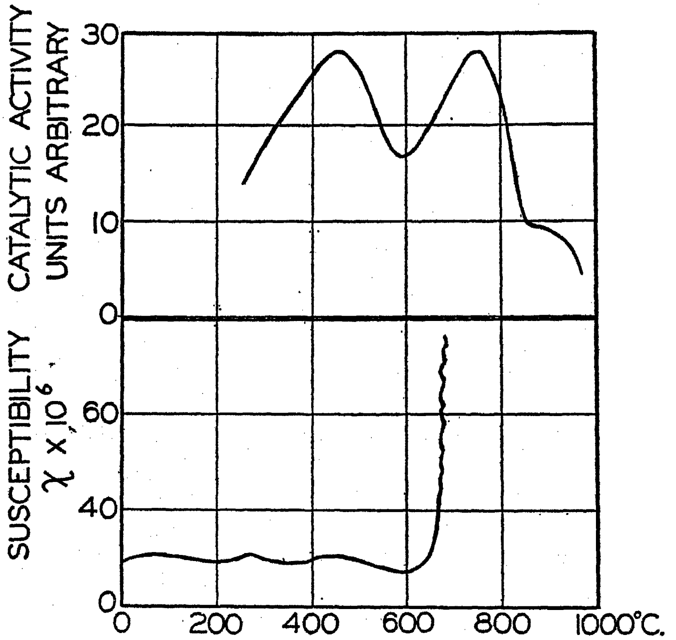


FIG. 13. Susceptibility and activity for the $CaO{-}Fe_2O_3$ system undergoing heating. The system becomes ferromagnetic after heating at 650°C.

Hüttig interprets the increased catalytic activity and the other changes as being due to an active intermediate compound. This is believed to form first on the surface of the particles, although it would seem more reasonable to suppose it formed at the interface between the particles of the two oxides. As heating proceeds the intermediate compound becomes larger in amount, but spinel formation also begins and soon destroys the intermediate. But by holding the temperature just below that at which spinel forms, Hüttig succeeds in preparing a mixture which he believes contains not less than 60 per cent of the active intermediate. This is indicated by a very great increase in catalytic activity. The intermediate is believed by Hüttig to be a compound of definite composition and properties, with a magnetic susceptibility of 51.5×10^{-6} at room temperature.

In spite of these assurances and of the very large number of papers published on so-called "active oxides," it must be admitted that the existence of these intermediate compounds can scarcely be regarded as established, by ordinary standards. But so far as the existence of an "active" *state* is concerned there can be no doubt, and Hüttig's work has produced a wealth of information concerning changes which take place in these systems.

The system $ZnO–Cr_2O_3$ (97, 106): This system, and the $CuO–Cr_2O_3$ system, are of particular interest in catalytic chemistry. The stoichiometric mixtures were prepared as usual, and ground for several hours. The reaction used for comparison of catalytic activity was the decomposition of methanol:

$$CH_3OH \rightarrow CO + 2H_2$$

After 6 hr. of heating at 400°C. the catalytic activity rose over 400 per cent. At the same time the magnetic susceptibility rose from an initial value of 23.4×10^{-6} (at 21°C.) to $> 65 \times 10^{-6}$ at low fields, and ferromagnetism appeared. Further heating reduced both catalytic activity and susceptibility, with simultaneous appearance of spinel formation in the x-ray diffraction diagram. The final product, after 12 hr. over the blast burner, yielded a spinel, inactive catalytically and with a susceptibility of 45.0×10^{-6} which was independent of field strength. It should be pointed out that development of ferromagnetism is not often found in chromium compounds free from elements which form paramagnetic ions. The changes described above are shown in figure 14.

The system $CuO–Cr_2O_3$ (104, 126): This system has been studied with reference to the decomposition of nitrous oxide. A rather obvious extension of all this work would be to compare the catalytic activity of the mixture on several different types of reactions to see if the heat treatment which produced maximum activity for one type of reaction was the same as that for another type. For the copper chromite system the catalytic activity reaches a low maximum after rather moderate heating, and then drops slowly and irregularly until spinel formation is complete after 6 hr. heating at 1000°C. During this time the magnetism rises slowly, then more rapidly from 22.6×10^{-6} to 34.2×10^{-6}. There is no indication of ferromagnetism, and no pronounced peak of catalytic activity. These changes are shown in figure 15.

The system $Cr_2O_3–Al_2O_3$ (104): Not very much information has been published on this system, but what little has been published is included here because of the considerable interest in this well-known cyclization catalyst. The data of Hüttig, Meyer, Kittel, and Cassirer unfortunately refer to α- rather than to γ-alumina. Stoichiometric mixtures of chromia and alumina, prepared mechanically in the usual way, have an average susceptibility of about 16.8×10^{-6}. This value is very slightly changed by prolonged heat treatment up to 1000°C. There is, of course, no expectation of spinel formation in this system, but the

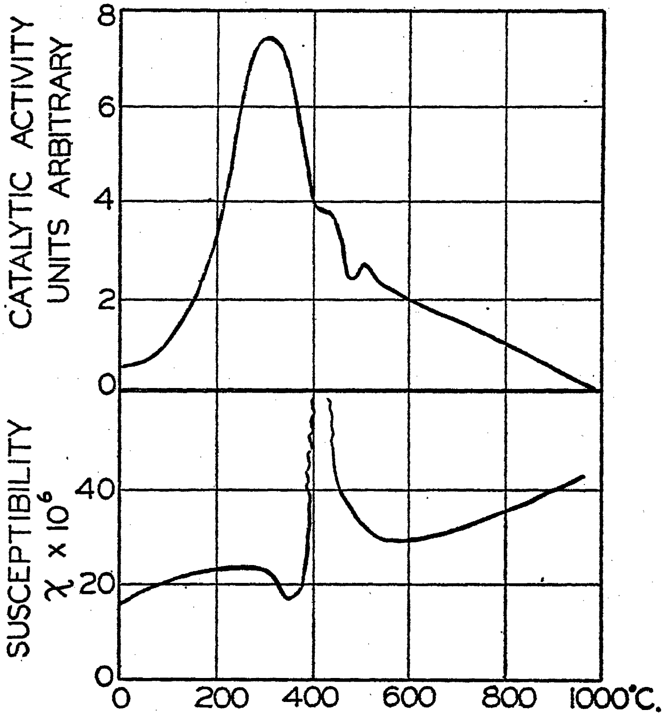


FIG. 14. Susceptibility and activity for the ZnO–Cr_2O_3 system undergoing heating. The system has a region of ferromagnetism at about 400°C.

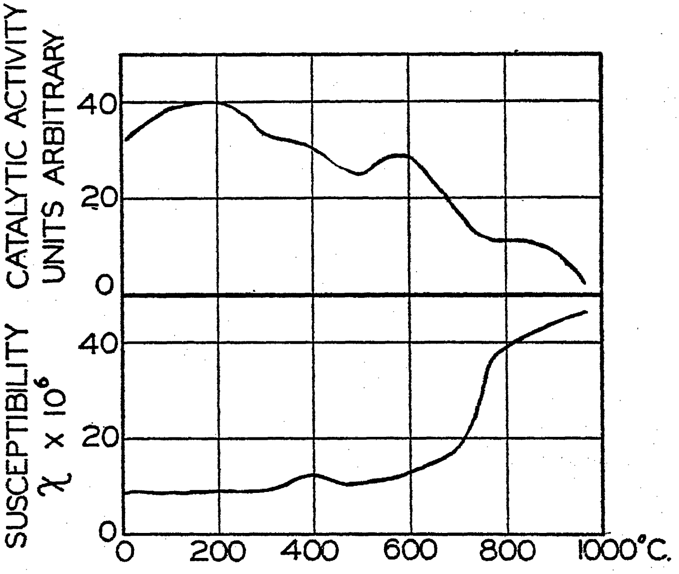


FIG. 15. Susceptibility and activity for the CuO–Cr_2O_3 system undergoing heating

system Fe_2O_3–Al_2O_3 shows a pronounced magnetic change after heat treatment in the neighborhood of 900°C. In this, and in all the other studies reported, the magnetic results would have been much more useful if they had been made at several temperatures. Some preliminary work by the writer suggests that a chromia–alumina catalyst prepared by the method of impregnating γ-alumina with chromium trioxide in solution, followed by reduction, gives a product with markedly different magnetic properties from the mechanical mixture of chromia plus alumina. These differences become very pronounced at liquid-air temperatures.

Other systems: In addition to those given above, the following systems have been subjected to magnetic and catalytic study by Hüttig and others.

Al_2O_3–Fe_2O_3 (104)
BaO–Fe_2O_3 (129)
BeO–Cr_2O_3 (126)
BeO–Fe_2O_3 (100, 101, 104, 109)
CdO–Cr_2O_3 (126)
CdO–Fe_2O_3 (127)
Cr_2O_3–Fe_2O_3 (104)
Cr_2O_3–SiO_2 (104)
Cr_2O_3–TiO_2 (104)
CuO–Al_2O_3 (104)
CuO–Fe_2O_3 (104, 127)
Fe_2O_3–SiO_2 (104)
Fe_2O_3–TiO_2 (104)
MgO–Al_2O_3 (109)
MgO–Cr_2O_3 (109, 130, 155)
MgO–Fe_2O_3 (50, 104, 105, 107, 108, 128, 131)
NiO–Fe_2O_3 (50)
PbO–Cr_2O_3 (126)
PbO–Fe_2O_3 (127)
SrO–Fe_2O_3 (129)
ZnO–Fe_2O_3 (93, 94, 95, 100, 103, 104, 109, 110)

IX. MAGNETISM AND ADSORPTION

The close relationship between adsorption and catalysis makes it necessary to say something about the former. Not very many magnetic studies have been made in this field, but what results are available are very interesting and could be profitably extended in several directions.

Determination of the valence states of dispersed substances on supported catalysts is an obvious application of magnetism to catalysis. For instance, Sachse (182) has used a ballistic method to determine the ferromagnetism of dispersed Fe_3O_4 on silica and on other supports. Adsorbed substances sometimes yield surprising results. In general the susceptibilities of systems involving adsorption do not obey the simple mixture law. Thus Bhatnagar, Mathur, and Kapur (17) have studied adsorption of the chlorides of iron, nickel, cobalt, and manganese on charcoal. These salts, all of which are normally strongly paramagnetic, become diamagnetic when they are adsorbed. Charcoal actually becomes more diamagnetic when it acts to adsorb such compounds. The charcoal must form definite covalent bonds to the metal ions, and the resulting system resembles the type of complex compound in which, through complete electron pairing, all paramagnetism is lost. This lack of additivity in adsorption is supported by some work of Boutaric and Berthier (28) on colloidal ferric hydroxide and on other substances.

The chemisorption of oxygen on metal oxides seems to have received magnetic study only from Bhatnagar *et al.*, on the compounds chromic oxide (12) and cobaltous oxide (19). Particularly in the case of the latter compound, the results show a diminution of susceptibility provided the adsorption is carried on above room temperature. This confirms the view that van der Waals adsorption cannot take place under these conditions, because molecular oxygen is very strongly paramagnetic. The cobalt may undergo surface oxidation to cobaltosic or cobaltic oxides.

The adsorption of oxygen on charcoal and on other relatively "inert" adsorbents has been studied by Aharoni and Simon (1) and by Juza and Langheim (120, 122). In spite of some differences of opinion it seems fairly well established that oxygen adsorbed on charcoal at room temperature has the same susceptibility as does gaseous oxygen. Courty (34) suggests that this may afford a convenient method of measuring the susceptibilities of gases. Courty's measurements appear not to have been carried out over a time or temperature interval, and it is here that the most interesting results are obtained. At room temperature, according to Juza and Langheim, the magnetic properties of adsorbed oxygen depend on time. The paramagnetism slowly diminishes owing, presumably, to chemical combination between the oxygen and the charcoal. It is possible to obtain the temperature coefficient of this reaction, and the activation energy of surface oxide formation works out to be about 5 kg.-cal. per mole.

At liquid-air temperatures the susceptibility of the oxygen depends, in addition, on the density of the adsorbed layer.

Similar systems which have been studied are palladium–hydrogen (1, 54, 122, 159, 188, 193), carbon–bromine (122), silica–bromine (122), and chabasite–oxygen (122).

The applications of such measurements to problems like the superficial oxidation and reduction of finely powdered metals are obvious.

One final observation in this connection may be made. There seems quite definitely to be an anomaly in the relationships between hydrogen and metallic nickel at the Curie temperature. Post and Ham (166) have shown that there is an abrupt discontinuity in the rate of hydrogen diffusion through nickel at the Curie point. Van Itterbeck, Mariens, and Verpoorten (206) have quite recently shown that the activated adsorption of hydrogen shows a sharp change at the Curie point (figure 16). It would be most interesting to know if these effects are part of a general phenomenon taking place with other gases and other metals. The possible relationships of such changes to the Hedvall effect are quite clear.

X. MAGNETIC SUSCEPTIBILITIES OF PURE SUBSTANCES OF CATALYTIC INTEREST

The following information covers the ionic diamagnetism of most elements and acid radicals, together with data on a variety of solids which find application in catalysis. While a fairly thorough search of the literature has been made, no attempt is made to give references to all the publications on each substance. Rather the values which seem most authoritative are given. Where it is impossible to choose between discordant values, both or all are generally given. It is hoped that these data will prove to be useful to readers who may wish to make magnetic studies. The data have already proved their value to the writer.

Ionic diamagnetism: The data given here (see table 3) are taken from the publications of Klemm (133–135). Other workers give other values, and it is not possible to decide which values are the most accurate (202). Those of Klemm are chosen because of their completeness and general coherence. These data will prove useful in making diamagnetic corrections. It should be pointed out that the diamagnetism of atoms and ions depends on the environment, but the changes are generally small. Figures are given on some paramagnetic ions. It must be emphasized that these data are the underlying diamagnetic correction factors, not the observable susceptibility.

Aluminum, Al: The susceptibility per gram of pure aluminum metal (3, 111, 214) at room temperature is about 0.62×10^{-6}. Note that aluminum is paramagnetic. The susceptibility is always slightly dependent on field strength and the above represents a value extrapolated to infinite field. However, quite large percentages of iron do not greatly alter this value, presumably owing to formation of the intermetallic compound $FeAl_3$. Treatment with sodium hydroxide decomposes this compound, liberating metallic iron, with a great increase of susceptibility (145). The susceptibility of pure aluminum is independent of temperature down to −170°C., and almost independent of temperature up to the melting point of the metal. The susceptibility is somewhat dependent on state of aggregation (220).

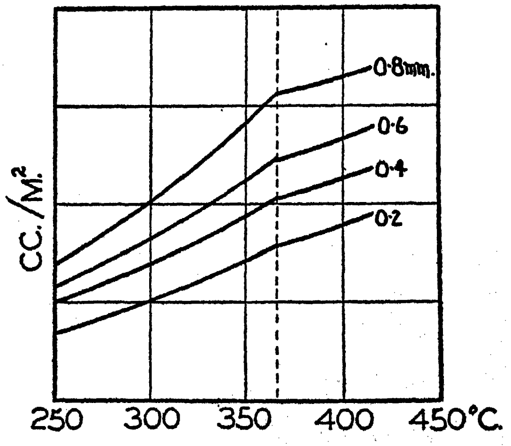


FIG. 16. Adsorption of hydrogen on nickel at several pressures in the neighborhood of the Curie point.

Aluminum oxide, Al_2O_3: The most complete study on alumina is that of Zimens (222). Pure γ-Al_2O_3 has $\chi = -0.34 \times 10^{-6}$ at room temperature. For α-Al_2O_3, $\chi = -0.27 \times 10^{-6}$. It is not clear if this slight difference may not in some way be related to the great change in specific surface which for γ-Al_2O_3 is 18.2 meters² per gram, and for α-Al_2O_3 is only 0.95 meters² per gram, as measured by Zimens by the emanation method. The two alumina hydrates, bayerite and böhmite, have a slightly greater diamagnetism than the oxide. The susceptibilities given, which are those for infinite field, are slightly dependent on field strength, presumably owing to impurities. Unfortunately no very

TABLE 3

Diamagnetic susceptibilities per gram-ion

Values in italics are for the underlying diamagnetism only of ions which may be paramagnetic

ION	$-\chi \times 10^6$	ION	$-\chi \times 10^6$	ION	$-\chi \times 10^6$
Ag^{+1}	24	Eu^{+3}	*20*	Os^{+4}	*29*
Ag^{+2}	*24*	F^{-1}	11	Os^{+6}	*18*
Al^{+3}	2	Fe^{+2}	*13*	Os^{+8}	11
As^{+3}	9?	Fe^{+3}	*10*	P^{+3}	4
As^{+5}	6	Ga^{+3}	8	P^{+5}	1
$\mathrm{AsO_3}^{-3}$	51	Ge^{+4}	7	$\mathrm{PO_3}^{-1}$	30
$\mathrm{AsO_4}^{-3}$	60	Gd^{+3}	*20*	$\mathrm{PO_3}^{-3}$	42
Au^{+1}	40?	Hf^{+4}	16	$\mathrm{PO_4}^{-3}$	50
Au^{+3}	32	Hg^{+2}	37	Pb^{+2}	28
B^{+3}	0.2	Ho^{+3}	*19*	Pb^{+4}	26
$\mathrm{BF_4}^{-1}$	39	I^{-1}	52	Pd^{+2}	25
$\mathrm{BO_3}^{-3}$	35	I^{+5}	12	Pd^{+4}	18
Ba^{+2}	32	I^{+7}	10	Pr^{+3}	*20*
Be^{+2}	0.4	$\mathrm{IO_3}^{-1}$	50	Pr^{+4}	*17*
Bi^{+3}	25?	$\mathrm{IO_4}^{-1}$	54	Pd^{+2}	*25*
Bi^{+5}	23	In^{+3}	19	Pd^{+4}	*18*
Br^{-1}	36	Ir^{+1}	*50*	Pt^{+2}	*40*
Br^{+5}	6	Ir^{+2}	*42*	Pt^{+3}	*33*
$\mathrm{BrO_3}^{-1}$	40	Ir^{+3}	*35*	Pt^{+4}	*28*
C^{+4}	0.1	Ir^{+4}	*29*	Rb^{+1}	20
CN^{-1}	18	Ir^{+6}	*20*	Re^{+3}	*36*
CNO^{-1}	21	K^{+1}	13	Re^{+4}	*28*
CNS^{-1}	35	La^{+3}	20	Re^{+6}	*16*
$\mathrm{CO_3}^{-2}$	34	Li^{+1}	0.6	Re^{+7}	12
Ca^{+2}	8	Lu^{+3}	17	Rh^{+3}	*22*
Cb^{+5}	9	Mg^{+2}	3	Rh^{+4}	*18*
Cd^{+2}	22	Mn^{+2}	*14*	Ru^{+3}	*23*
Ce^{+3}	*20*	Mn^{+3}	*10*	Ru^{+4}	*18*
Ce^{+4}	17	Mn^{+4}	*8*	S^{-2}	38?
Cl^{-1}	26	Mn^{+6}	*4*	S^{+4}	3
Cl^{+5}	2	Mn^{+7}	3	S^{+6}	1
$\mathrm{ClO_3}^{-1}$	32	Mo^{+2}	*31*	$\mathrm{SO_3}^{-2}$	38
$\mathrm{ClO_4}^{-1}$	34	Mo^{+3}	*23*	$\mathrm{SO_4}^{-2}$	40
Co^{+2}	*12*	Mo^{+4}	*17*	$\mathrm{S_2O_3}^{-2}$	49
Co^{+3}	*10*	Mo^{+5}	*12*	$\mathrm{S_2O_8}^{-2}$	78
Cr^{+2}	*15*	Mo^{+6}	7	Sb^{+3}	17?
Cr^{+3}	*11*	N^{+5}	0.1	Sb^{+5}	14
Cr^{+4}	*8*	$\mathrm{NH_4}^{+1}$	11.5	Sc^{+3}	6
Cr^{+5}	*5*	$\mathrm{NO_2}^{-1}$	10	Se^{-2}	48?
Cr^{+6}	3	$\mathrm{NO_3}^{-1}$	20	Se^{+4}	8
Cs^{+1}	31	Na^{+1}	5	Se^{+6}	5
Cu^{+1}	12	Nd^{+3}	*20*	$\mathrm{SeO_3}^{-2}$	44
Cu^{+2}	*11*	Ni^{+2}	*12*	$\mathrm{SeO_4}^{-2}$	51
Dy^{+3}	*19*	OH^{-1}	12	Si^{+4}	1
Er^{+3}	*18*	Os^{+2}	*44*	$\mathrm{SiO_3}^{-2}$	36
Eu^{+2}	*22*	Os^{+3}	*36*	Sm^{+2}	*23*

TABLE 3—*Concluded*

ION	$-\chi \times 10^6$	ION	$-\chi \times 10^6$	ION	$-\chi \times 10^6$
Sm^{+3}	*20*	Ti^{+2}	*15*	V^{+5}	4
Sn^{+2}	20	Ti^{+3}	*9*	W^{+2}	*41*
Sn^{+4}	16	Ti^{+4}	5	W^{+3}	*36*
Sr^{+2}	15	Tl^{+1}	34	W^{+4}	*23*
Ta^{+5}	14	Tl^{+3}	31	W^{+5}	*19*
Tb^{+3}	*19*	Tm^{+3}	*18*	W^{+6}	13
Tb^{+4}	*17*	U^{+3}	*46*	Y^{+3}	12
Te^{-2}	70?	U^{+4}	*35*	Yb^{+2}	*20*
Te^{+4}	14	U^{+5}	*26*	Yb^{+3}	*18*
Te^{+6}	12	U^{+6}	19	Zn^{+2}	10
$\mathrm{TeO_3}^{-2}$	63	V^{+2}	*15*	Zr^{+4}	10
$\mathrm{TeO_4}^{-2}$	55	V^{+3}	*10*		
Th^{+4}	23	V^{+4}	*7*		

accurate measurements appear to have been made on "pure" aluminum oxide at low temperatures. The writer's experience is that material which is diamagnetic at room temperature invariably becomes paramagnetic at −190°C., owing to paramagnetic impurities being present. "Pure" aluminum oxide would, presumably, have a susceptibility independent of both field strength and temperature.

Asbestos: No magnetic measurements appear to have been made on asbestos. Untreated asbestos would invariably contain appreciable iron as impurity. "Pure" asbestos would probably be diamagnetic.

Beryllia, BeO: Hüttig and Kittel give several values, averaging $\chi = -0.1 \times 10^{-6}$ at room temperature. This susceptibility is essentially independent of field strength. No data at low temperatures are available. "Pure" beryllium oxide should have no temperature dependence of susceptibility.

Boric oxide, B_2O_3: The only measurement on this substance appears to be that of Meyer, $\chi = -0.55 \times 10^{-6}$ at 14°C., as quoted in the *International Critical Tables* (112).

Charcoal, C: It is surprising that after many years of magnetochemical research there are available no measurements on relatively pure activated charcoal. Several measurements by the writer on charcoals of varying degrees of activity all show strong paramagnetism or ferromagnetism, owing to impurities. "Pure" charcoal should be diamagnetic. The susceptibility is probably about -0.4×10^{-6}. Graphite has a very large magnetic anisotropy and a correspondingly large diamagnetism for the powdered substance. Magnetic measurements on activated charcoal are further complicated by the possibility of "surface" paramagnetism, and of adsorbed gases.

Calcium oxide, CaO: Kittel and Hüttig (130) give $\chi = -0.58 \times 10^{-6}$ at room temperature. The data of these workers are not too reliable and it is possible that the older susceptibility of Meyer (113) is more accurate. Meyer gives $\chi = -0.27 \times 10^{-6}$. No measurements are available at low temperatures.

"Pure" calcium oxide should have no temperature coefficient of susceptibility.

Cadmium oxide, CdO: Meyer (153) gives $\chi = -0.30 \times 10^{-6}$ at 15°C.

Cerium oxide, CeO_2: Williams (217) gives $\chi = 0.14 \times 10^{-6}$ at 20°C. Ceric oxide is an example of a substance which has a small temperature-independent paramagnetism, although it must be admitted that there have not been many measurements made on material of established purity. Cerous oxide, Ce_2O_3, seems to have escaped magnetochemical investigation. It should be fairly strongly paramagnetic with the usual large temperature coefficient. The valence state of cerium in a solid is easily found by magnetic measurements.

Cobalt, Co: All the ferromagnetic metals have been subjected to innumerable magnetic studies. For our present purpose two quantities only are required, the specific magnetization and the Curie point. These both depend to a marked degree on purity and on other circumstances. We shall give $\sigma = 165$ and the Curie temperature 1100°C. (144).

Oxides of cobalt: A careful study has been made by Bhatnagar, Prakash, and Qayyum (20). The susceptibilities depend slightly on mode of preparation, but if care is taken to insure purity and identity the results may readily be duplicated. The data of these workers on the three common cobalt oxides are summarized below:

	CoO	Co_3O_4	Co_2O_3
$\chi \times 10^6$	68.4	30.7	28.8
Δ	315°	153°	180°

The susceptibilities of these substances follow the Weiss law, with Δ values as shown. The measurements were actually made between room temperature and about 500°C. The oxide Co_3O_4 becomes ferromagnetic after long heating at high temperature.

Cobalt sulfide, CoS_2: Haraldsen and Klemm have studied the system cobalt–sulfur. The compound CoS_2 has a susceptibility of 30.5×10^{-6} at room temperature. There are large and interesting variations of susceptibility with sulfur content, yielding in some cases ferromagnetism.

Chromic oxide, Cr_2O_3: This important oxide, from the catalytic standpoint, has been the subject of many magnetic studies. The work of Bhatnagar, Cameron, Harbord, Kapur, King, and Prakash (12) is the most complete, and brings some order out of a rather chaotic situation. Chromic oxide prepared in a large variety of ways has nearly the same susceptibility. The oxide prepared by ignition of the precipitated hydroxide has the values:

°K	302	325.5	335.5	355.5	395	464.5	540	615
$\chi \times 10^6$	25.86	26.71	26.94	26.40	26.02	24.78	22.40	20.78

Chromic oxide prepared by ignition of chromium trioxide has substantially the same susceptibility. There is some evidence that the susceptibility of chromic oxide rises sharply at still higher temperatures, but this may be due to decomposition. At lower temperatures the older data of Honda and Soné still seem to be satisfactory (84). They give the following: at 18°C. $\chi = 25.5 \times 10^{-6}$; at −64°C. $\chi = 22.2 \times 10^{-6}$; at −186°C. $\chi = 20.1 \times 10^{-6}$. It will be noticed that the temperature coefficient of susceptibility is small, and is opposite in sign from that predicted by the Curie law, at least at low temperatures. The susceptibility of the active oxide gel produced through slow interaction of dilute chromic nitrate and ammonia is not sensibly different from that reported for the crystalline oxide (203). The anomalous temperature coefficient is believed to be due in part to interaction between the chromium atoms. When chromic oxide is heated in hydrogen at 440°C. a drop in susceptibility occurs. This amounts to 4 or 5 per cent (12). The change is reversed by heating in oxygen. Strong heating of chromic oxide in hydrogen yields ferromagnetic oxides. There also apparently occur a series of ferromagnetic oxides approaching the formula Cr_5O_9. However, Michel and Benard (158) claim that there is a definite ferromagnetic compound CrO_2, isomeric with a non-ferromagnetic $CrO_3 \cdot Cr_2O_3$. The ferromagnetic substance has a Curie point at 116°C., and is best prepared through decomposition of chromyl chloride. This substance was not obtained pure.

Chromic anhydride, CrO_3: Grey and Dakers (55) and Tilk and Klemm (200) show that this substance has a small temperature-independent paramagnetism, with $\chi = 0.4 \times 10^{-6}$.

The chromium–sulfur system: The compound CrS is paramagnetic, according to Haraldsen and Kowalski (66), with a susceptibility at room temperature of 20.0×10^{-6}. This is almost independent of temperature. Chromic sulfide, Cr_2S_3, is also paramagnetic with about the same susceptibility. But between these compositions there are some very interesting ferromagnetic systems. The ratio $CrS_{1.17}$ is strongly ferromagnetic between the temperatures 165°K. and 310°K.

Alkali thiochromites, $NaCrS_2$, $KCrS_2$: The use of sulfides and possible thio salts as catalysts makes it appropriate to mention the recent work of Rüdorff and Stegemann (181) on alkali thiochromites. Several different preparations gave reasonably consistent results. At room temperature for $NaCrS_2$, $\chi = 45 \times 10^{-6}$, and for $KCrS_2$, $\chi = 72 \times 10^{-6}$. Both salts follow the Weiss law: for $NaCrS_2$, $\Delta = -37°$; for $KCrS_2$, $\Delta = -116°$. The potassium salt becomes ferromagnetic at liquid-air temperature. These results are quite different from those obtained on Cr_2S_3. Rüdorff and Stegemann relate these differences to the distances between chromium atoms in the compounds, which are as follows:

COMPOUND	Cr–Cr DISTANCE
	Å.
Cr_2S_3	2.78–3.42
$NaCrS_2$	3.53
$KCrS_2$	3.62

This is a good example of how the magnitude of the quantity Δ in the Weiss law may sometimes be used to estimate the proximity and degree of interaction of the magnetic atoms or ions in a compound.

Copper, Cu: Metallic copper (82) is slightly diamagnetic with a susceptibility of -0.08×10^{-6}. This value is substantially independent of temperature. Considerable difficulty is encountered in obtaining copper sufficiently free from ferromagnetic impurities to show no dependence of susceptibility on field strength. Corrections may be made in the usual way by extrapolating the susceptibility to infinite field. Morris and Selwood (162) describe methods for preparing "magnetically pure" copper. The impurities may sometimes be forced into solution by quenching the copper from a high temperature, but such procedures are seldom feasible with material prepared for catalytic purposes. The influence of particle size on the susceptibility of copper has been studied by Bhatnagar, Verma, and Anwar-ul-Haq (23).

Cuprous oxide, Cu_2O: This compound is diamagnetic, according to Klemm and Schüth (137) and Bhatnagar and Mitra (18). The susceptibility is -0.18×10^{-6}. The Indian experimenters state that superficial oxide films on copper are always paramagnetic, owing to the presence of cupric oxide. The general belief has been that such films consisted solely of cuprous oxide.

Cupric oxide, CuO: Klemm and Schüth (137) give the following data:

Temperature, °C.	20	100	235	330	445
$\chi \times 10^6$	2.88	3.06	3.20	3.16	3.10

Different preparations have rather different susceptibilities, but all show the peculiar temperature coefficient. No measurements at low temperatures have come to the writer's attention. These would be useful in the study of copper and copper-containing catalysts.

Copper sulfides, Cu_2S (153) and CuS (137): Both these substances are diamagnetic. For Cu_2S, $\chi = -0.18 \times 10^{-6}$; for CuS, $\chi = -0.02 \times 10^{-6}$. This illustrates the rather frequent occurrence of diamagnetism in sulfides for which the corresponding oxide is paramagnetic.

Gold, Au: Metallic gold (82) is diamagnetic with a susceptibility of -0.15×10^{-6}, substantially independent of temperature.

Hafnium oxide, HfO_2: This substance is stated by Meyer (152) to be diamagnetic, with a susceptibility of -0.110×10^{-6}. This value is not surprising, although the presence of a small temperature-independent paramagnetism might be expected.

Iron, Fe: Metallic iron is strongly ferromagnetic. The magnetic properties depend in great degree on the purity and previous history of the sample. These changes have been the subject of almost innumerable researches. The specific magnetization is about 220, and the Curie point about 760°C.

Ferrous oxide, FeO: Welo and Baudisch (147) state that ferrous oxide hydrate has a susceptibility at room temperature of about 20×10^{-6}. Bizette and Tsai (25) have recently reported that the susceptibility has a maximum at 198°K., and that between 293°K. and 213°K., $\chi = 6.24/(T + 570°)$. No other information on this compound seems to be available.

Ferric oxide, Fe_2O_3: There are at least two compounds of this formula (147).

γ-Fe_2O_3 is ferromagnetic. Its magnetic properties appear to be almost identical with those of magnetite, Fe_3O_4. The Curie point is reported by various authors to lie between 500°C. and 620°C. The specific magnetization is about the same as that of nickel, namely $\sigma = 50$–60. α-Fe_2O_3 is, on the other hand, paramagnetic, or more probably very weakly ferromagnetic (68). The susceptibility at room temperature is about 22×10^{-6}, but varies irregularly with temperature, becoming field dependent as the temperature is lowered. Different preparations show widely different magnetic behavior, but this may be due to failure accurately to characterize the substances on which measurements have been made. The susceptibility also varies with grain size (30).

Magnetite, Fe_3O_4: This substance is ferromagnetic, about to the same degree as nickel. The Curie point is sometimes given as 590°C., but this varies widely in different preparations (147, 148).

Iron–sulfur system: In general, low sulfur concentrations lead to paramagnetism and high to ferromagnetism. But this is not invariably true. The iron–sulfur system is extremely complex and, at its present state of development, not particularly useful from a magnetocatalytic standpoint. This system has been extensively studied by Haraldsen and others (58–61, 63, 119, 142).

Kaolin: Zimens and Hedvall (221) give χ as about 0.2×10^{-6}, but unfortunately it is not clear from this paper whether the susceptibility is negative or positive. As with all natural substances, iron may be an important impurity.

Magnesium, Mg (81): The metal has a small, temperature-independent paramagnetism, $\chi = 0.55 \times 10^{-6}$. As with most metals, correction for ferromagnetic impurity is practically always necessary. Rao and Savithri (175) give the much higher susceptibility 1.08×10^{-6}. It is not clear to what this difference may be due.

Magnesium oxide, MgO: The value given by Meyer (152) is probably close to the truth. The susceptibility is -0.25×10^{-6}, and is independent of temperature.

Manganese, Mn: Pantulu (170) gives the susceptibility of the amorphous metal at 20°C. as 11.80×10^{-6}. The susceptibility follows the Weiss law with $\Delta = 1540°$. Other investigators give values for χ in the range 8–11×10^{-6}. Wheeler reports a difference in susceptibility of α- and β-manganese (215). Manganese appears to form ferromagnetic compounds with nitrogen at quite moderate temperatures (5).

Manganous oxide, MnO: Haraldsen and Klemm (65) give the following:

T, °K.	90	195	293	523	683
$\chi \times 10^6$	73.0	75.7	69.1	53.4	46.5

Bhatnagar (12) *et al.* give results about 5 per cent higher, but with approximately the same temperature coefficient.

Manganosic oxide, Mn_3O_4: Like other oxides of similar formula, this substance is not very well characterized. Honda and Soné (85) give the susceptibility at room temperature as 55.8×10^{-6}

Manganic oxide, Mn_2O_3: Bhatnagar (12) *et al.* give the susceptibility at 20°C. as 89.3×10^{-6}. This value is considerably higher than those given in earlier reports.

Manganese dioxide, MnO_2: Bhatnagar (12) *et al.* have carefully investigated this important compound. Their results are given below:

T, °K	300	310	340	383	438	480
$\chi \times 10^6$	26.0	25.7	24.8	23.6	22.2	21.3

Manganous sulfide, MnS: Haraldsen and Klemm (65) give the following data:

T, °K	90	195	293	528	726
$\chi \times 10^6$	67.3	73.6	64.7	48.5	40.3

However, MnS exists in at least two forms which have somewhat different susceptibilities (199).

Manganese disulfide, MnS_2: Haraldsen and Klemm (64) give the following data:

T, °K	90	195	290	423
$\chi \times 10^6$	60	53	47	40

These data are, however, only approximate, because some dependence of susceptibility on field strength was found.

Oxides of molybdenum: These compounds are not in a very satisfactory state, magnetically speaking. For Mo_2O_3 Meyer (152) gives $\chi = -0.35 \times 10^{-6}$ at 16°C. This result is rather surprising, because an appreciable paramagnetism would have been predicted. The compound MoO_2 is given a susceptibility of $+0.33 \times 10^{-6}$ by Wedekind and Horst (212). Molybdic anhydride, MoO_3, might be expected to have a small temperature-independent susceptibility, and this appears to be the case (8, 201, 212), although values range from 0.876×10^{-6} to 0.02×10^{-6}.

Sulfides of molybdenum: The compounds MoS_2 and MoS_3 are diamagnetic with susceptibilities of -0.48×10^{-6} and -0.36×10^{-6}, respectively (154, 201). It would be interesting to know why oxides often show temperature-independent paramagnetism while the corresponding sulfides do not.

Nickel, Ni: From the very large number of reports on metallic nickel it is safe to say that the specific magnetization is about 55 and that the Curie point is about 358°C.

Nickel oxide, NiO: For a long time the commonly accepted susceptibility for this substance at room temperature was about 50×10^{-6}. Klemm (136, 138) has, however, shown that nickel oxide as ordinarily prepared is ferromagnetic, owing to the presence of metallic nickel. Only when the proportion of oxygen exceeds that in the stoichiometric ratio of NiO does the oxide become paramagnetic. The identity of the ferromagnetism with the presence of metallic nickel is proved by the fact that the ferromagnetism disappears above the Curie point of pure nickel. Bhatnagar and Bal (11) show that when care is taken in the preparation, nickel oxide of proper stoichiometric proportions may be made, and that the susceptibility is 9.56×10^{-6} at room temperature.

Nickelous sulfide, NiS: This substance is not particularly easy to characterize accurately. A sample analyzing $NiS_{1.015}$ is stated by Klemm and Schüth (138) to have a susceptibility of about 3×10^{-6} at 20°C. and 2000 oersteds. There is a marked dependence on field strength and also on temperature. It will be noted that this susceptibility is considerably smaller than that for the corresponding oxide.

Nickel disulfide, NiS_2: Haraldsen and Klemm (64) find that at 20°C. and extrapolated to infinite field, $\chi = 5.1 \times 10^{-6}$. There is, however, some dependence on field strength. Temperature dependence is also shown.

Platinum, Pt: The metal has been investigated extensively. The susceptibility at room temperature is about 1.0×10^{-6}, with a small temperature coefficient (210). Takatori (194) reports a decreased susceptibility for finely powdered platinum.

Rhenium, Re: Perakis and Capatos (173) give the susceptibility as $+0.369 \times 10^{-6}$, and as independent of temperature from $-79°$ to 20°C. The value at room temperature has recently been confirmed by Miss Marylinn Ellis in the writer's laboratory.

Rhenium oxides and sulfides: Schüth and Klemm (184) give the following susceptibilities at room temperature:

Compound..............	ReO_2	ReS_2	ReO_3	Re_2O_7
$\chi \times 10^6$..............	+0.2	−0.15	+0.15	−0.06

All these values are substantially independent of temperature.

Silica, SiO_2: Pascal (171) gives $\chi = -0.493 \times 10^{-6}$. This author has also studied the susceptibility of silica as a function of water content.

Silver, Ag: Metallic silver (151, 161, 175) is diamagnetic, $\chi = -0.19 \times 10^{-6}$.

Strontium oxide, SrO: Meyer (151) gives $\chi = -0.060 \times 10^{-6}$. This value seems a little small.

Sulfur, S: Zimens and Hedvall (221) give $\chi = -0.45 \times 10^{-6}$. Both rhombic and monoclinic sulfur have about the same susceptibility.

Thorium oxide, ThO_2: Bourion and Beau (26) state that anhydrous ThO_2 is feebly paramagnetic but no data are given. Miss Ellis in the writer's laboratory finds $\chi = -0.1 \times 10^{-6}$ at room temperature.

Tin oxides (154): Both SnO and SnO_2 are diamagnetic, with $\chi = -0.11$ and -0.05×10^{-6}, respectively.

Titanium dioxide, TiO_2: Zimens and Hedvall (221) have made a careful study of titanium oxide and of its hydrates. Pure "synthetic" rutile has a susceptibility of 0.13×10^{-6}, anatase a value slightly lower, perhaps about 0.09×10^{-6}. Hydrated TiO_2 (16.5 per cent water) is diamagnetic, $\chi = -0.05 \times 10^{-6}$. All these values are independent of field strength. They would be more useful if the measurements had been extended to low temperatures. The system titanium–oxygen has been studied by Ehrlich (39, 40).

Tungstic oxide, WO_3: $\chi = +0.81 \times 10^{-6}$, according to Wedekind and Horst (213). In view of the interest in tungsten compounds as catalysts, it is unfortunate that more magnetic data are not available.

Oxides of uranium: Haraldsen and Bakken (62) give the following susceptibilities at room temperature:

COMPOUND	$\chi \times 10^6$
UO_3	+0.55
UO_2	1.7
U_3O_8	0.4

There is some doubt as to the identity of U_3O_8. Both UO_2 and U_3O_8 follow the Weiss law with $\Delta = 180°$ and $170°$, respectively.

Vanadium oxides and sulfides: Hoschek and Klemm (86) give the following:

COMPOUND	$\chi \times 10^6$		
	20°C.	−78°C.	−183°C.
V_2O_5	0.35	0.38	0.49
VO_2	0.74	0.71	0.80
V_2O_3	12.2	13.3	8.7
V_2S_3	7.9	10.1	20.3

The system vanadium–oxygen (38, 87) has been further studied, with some very peculiar results in the neighborhood of VO_2. The sulfide V_2S_5 probably does not exist. A substance analyzing to this formula is strongly paramagnetic, a result which is improbable for pentavalent vanadium.

Zinc oxide, ZnO: This oxide is diamagnetic, but the value seems to depend on previous heat treatment. Hüttig, Radler, and Kittel (106) report susceptibilities from -0.29 to -0.43×10^{-6}, depending on temperature of ignition. Turkevich and Selwood (204) found zinc oxide to be paramagnetic at liquid-air temperatures unless it was first ignited strongly.

Zirconia, ZrO_2: Bourion and Hun (27) state that anhydrous ZrO_2 is slightly diamagnetic, but no data are given.

REFERENCES

(1) Aharoni and Simon: Z. physik. Chem. **B4**, 175 (1929).
(2) Aoyama, Matsuzawa, and Tokahashi: Sci. Papers Inst. Phys. Chem. Research (Tokyo) **34**, 957 (1938).
(3) Auer: Z. Physik **92**, 283 (1934).
(4) Bachmann: In *Organic Chemistry* (H. Gilman, *Editor*), Vol. I, p. 618. John Wiley and Sons, Inc., New York (1943).
(5) Bates, Gibbs, and Pantulu: Proc. Phys. Soc. (London) **48**, 665 (1936).
(6) Baudisch: Ber. **68B**, 769 (1935).
(7) Baudisch and Welo: Chem.-Ztg. **49**, 661 (1925).
(8) Berkman and Zocher: Z. physik. Chem. **124**, 318 (1926).
(9) Berlaga and Gorskiĭ: J. Exptl. Theoret. Phys. (U.S.S.R.) **4**, 522 (1934).
(10) Berndt: Physik. Z. **9**, 512 (1908).
(11) Bhatnagar and Bal: J. Indian Chem. Soc. **11**, 603 (1934).

(12) BHATNAGAR, CAMERON, HARBARD, KAPUR, KING, AND PRAKASH: J. Chem. Soc. **1939,** 1433.
(13) BHATNAGAR, KAPUR, BHATNAGAR, AND QAYYUM: J. Indian Chem. Soc. **18,** 391 (1941).
(14) BHATNAGAR, KAPUR, BHATNAGAR, AND PRAKASH: J. Indian Chem. Soc. **18,** 371 (1941).
(15) BHATNAGAR, MATHUR, AND KAPUR: Phil. Mag. [7] **8,** 457 (1929).
(16) BHATNAGAR AND MATHUR: *Physical Principles and Applications of Magnetochemistry.* Macmillan and Company, Ltd., London (1935).
(17) BHATNAGAR, MATHUR, AND KAPUR: Indian J. Phys. **3,** 53 (1928).
(18) BHATNAGAR AND MITRA: Current Sci. **1,** 343 (1933).
(19) BHATNAGAR, PRAKASH, AND QAYYUM: J. Indian Chem. Soc. **18,** 540 (1941).
(20) BHATNAGAR, PRAKASH, AND QAYYUM: J. Indian Chem. Soc. **18,** 540 (1941).
(21) BHATNAGAR, PRAKASH, AND SINGH: J. Indian Chem. Soc. **17,** 125 (1940).
(22) BHATNAGAR, PRAKASH, AND SINGH: J. Indian Chem. Soc. **17,** 133 (1940).
(23) BHATNAGAR, VERMA, AND ANWAR-UL-HAQ: Kolloid-Z. **78,** 9 (1937).
(24) BHATNAGAR, YAJNIK, KAPUR, AND BHATNAGAR: J. Indian Chem. Soc. **18,** 350 (1941).
(25) BIZETTE AND TSAI: Compt. rend. **217,** 390 (1943).
(26) BOURION AND BEAU: Compt. rend. **198,** 916 (1934).
(27) BOURION AND HUN: Compt. rend. **187,** 886 (1928).
(28) BOUTARIC AND BERTHIER: J. Chem. Phys. **39,** 129 (1942).
(29) BOZORTH: J. Optical Soc. Am. **10,** 591 (1925).
(30) CHEVALLIER AND MATHIEU: Ann. phys. **18,** 258 (1943).
(31) CLARK AND ARCHIBALD: Trans. Roy. Soc. Canada [3] **24,** Sec. III, 121 (1930).
(32) COHN AND HEDVALL: J. Phys. Chem. **46,** 841 (1942).
(33) COHN: Svensk. Kem. Tid. **52,** 49 (1940).
(34) COURTY: Compt. rend. **216,** 769 (1943).
(35) CREMER: *Handbuch der Katalyse* (G.-M. Schwab, *Editor*) Vol. 1, p. 325. Springer-Verlag, Wien (1941).
(36) DE HEMPTINNE: Z. physik. Chem. **34,** 669 (1900).
(37) DRABKIN: Ann. Rev. Biochem. **11,** p. 531. Stanford University Press, Stanford University, California (1942).
(38) DRESSNANDT AND SCHOTTKY: Naturwissenschaften **27,** 840 (1939).
(39) EHRLICH: Z. Elektrochem. **45,** 362 (1939).
(40) EHRLICH: Z. anorg. allgem. Chem. **247,** 53 (1941).
(41) ELEY: Trans. Faraday Soc. **36,** 500 (1940).
(42) ELMORE: Phys. Rev. **54,** 1092 (1938).
(43) FARQUHARSON AND ADY: Nature **143,** 1067 (1939).
(44) FARKAS: *Orthohydrogen, Parahydrogen, and Heavy Hydrogen.* Cambridge University Press, Cambridge (1935).
(45) FARKAS AND SACHSSE: Trans. Faraday Soc. **30,** 331 (1934).
(46) FISCHBECK, NEUNDEUBEL, AND SALZER: Z. Elektrochem. **40,** 517 (1934).
(47) FISCHBECK AND SALZER: Z. Elektrochem. **41,** 158 (1935).
(48) FORESTIER *et al*: Compt. rend. **190,** 1421 (1930); **195,** 954 (1932); **197,** 54 (1933); **204,** 265, 1254 (1937); **208,** 1891 (1939).
(49) FORESTIER AND LILLE: Compt. rend. **204,** 265, 1254 (1937); **208,** 1891 (1939).
(50) FORESTIER AND VETTER: Compt. rend. **209,** 164 (1939).
(51) FREED AND THODE, quoted by PITZER: J. Am. Chem. Soc. **67,** 1131 (1945).
(52) GARNER AND JACKMAN: J. Chem. Soc. **119,** 1936 (1921); **121,** 1298 (1923).
(53) GILBERT, TURKEVICH, AND WALLIS: J. Org. Chem. **3,** 611 (1939).
(54) GRAHAM: Ann. chim. phys. **16,** 197 (1869).
(55) GREY AND DAKERS: Phil. Mag. **11,** 237 (1931).
(56) GRUBE AND WINKLER: Z. Elektrochem. **41,** 52 (1935).
(57) HALLER AND SELWOOD: J. Am. Chem. Soc. **61,** 85 (1939).
(58) HARALDSEN: Z. anorg. allgem. Chem. **231,** 78 (1937).
(59) HARALDSEN: Tids. Kjemi Bergvesen **19,** 144 (1939).

(60) Haraldsen: Z. anorg. allgem. Chem. **246,** 169 (1941).
(61) Haraldsen: Z. anorg. allgem. Chem. **246,** 195 (1941).
(62) Haraldsen and Bakken: Naturwissenschaften **28,** 127 (1940).
(63) Haraldsen and Klemm: Z. anorg. allgem. Chem. **223,** 209 (1935).
(64) Haraldsen and Klemm: Z. anorg. allgem. Chem. **223,** 409 (1935); **224,** 85 (1935).
(65) Haraldsen and Klemm: Z. anorg. allgem. Chem. **220,** 183 (1934).
(66) Haraldsen and Kowalski: Z. anorg. allgem. Chem. **224,** 329 (1935).
(67) Haul and Schoon: Z. Elektrochem. **45,** 663 (1939).
(68) Hayes: U. S. Bur. Mines, Repts. Investigations Tech. Papers, No. 3570 (1941).
(69) Hedvall: *Reaktionsfähigkeit fester Stoffe.* J. A. Barth, Leipzig (1938).
(70) Hedvall: Z. Elektrochem. **41,** 445 (1935).
(71) Hedvall: Angew. Chem. **54,** 505 (1941).
(72) Hedvall: Atti X° Congr. intern. Chim. **2,** 255 (1938).
(73) Hedvall and Berg: Z. physik. Chem. **B41,** 388 (1938).
(74) Hedvall and Byström: Z. physik. Chem. **B41,** 163 (1938).
(75) Hedvall and Gustavson: Svensk. Kem. Tid. **46,** 64 (1934).
(76) Hedvall and Hedin: Z. physik. Chem. **B30,** 280 (1935).
(77) Hedvall, Hedin, and Persson: Z. physik. Chem. **B27,** 196 (1934).
(78) Hedvall and Sanford: Z. physik. Chem. **B29,** 455 (1935).
(79) Hedvall: Svensk. Kem. Tid. **47,** 161 (1935).
(80) Henglein: Z. Elektrochem. **32,** 213 (1926).
(81) Honda: *International Critical Tables,* Vol. VI, p. 355. McGraw-Hill Book Company, Inc., New York (1929).
(82) Honda and Owen: *International Critical Tables,* Vol. VI, p. 354. McGraw-Hill Book Company, Inc., New York (1929).
(84) Honda and Soné: *International Critical Tables,* Vol. VI, p. 358. McGraw-Hill Book Company, Inc., New York (1929).
(85) Reference 84, p. 357.
(86) Hoschek and Klemm: Z. anorg. allgem. Chem. **226,** 359 (1936).
(87) Hoschek and Klemm: Z. anorg. allgem. Chem. **242,** 63 (1939).
(88) Hüttig: Kolloid-Z. **99,** 262 (1942).
(89) Hüttig: Kolloid-Z. **98,** 263 (1942).
(90) Hüttig: Kolloid-Z. **97,** 281 (1941).
(91) Hüttig: Kolloid-Z. **94,** 258 (1941).
(92) Hüttig: Kolloid-Z. **94,** 137 (1941).
(93) Hüttig: Angew. Chem. **49,** 882 (1936).
(94) Hüttig: Z. Elektrochem. **44,** 571 (1938).
(95) Hüttig: J. chim. phys. **36,** 84 (1939).
(96) Hüttig: Naturwissenschaften **20,** 640 (1932)
(97) Hüttig, Cassirer, and Strotzer: Z. Elektrochem. **42,** 215 (1936).
(98) Hüttig, Ehrenberg, and Kittel: Z. anorg. allgem. Chem. **228,** 112 (1936).
(99) Hüttig, Funke, and Kittel: J. Am. Chem. Soc. **57,** 2470 (1935).
(100) Hüttig, Geisler, Hampel, Hnevkovsky, Jeitner, Kittel, Kostelitz, Owesny, Schmeiser, Schneider, and Sedlatschek: Z. anorg. allgem. Chem. **237,** 209 (1938).
(101) Hüttig and Kittel: Gazz. chim. ital. **63,** 833 (1933).
(102) Hüttig and Kittel: Gazz. chim. ital. **63,** 834 (1933).
(103) Hüttig, Kittel, and Herrmann: Z. anorg. allgem. Chem. **210,** 26 (1933).
(104) Hüttig, Meyer, Kittel, and Cassirer: Z. anorg. allgem. Chem. **224,** 225 (1935.)
(105) Hüttig, Novak-Schreiber, and Kittel: Z. physik. Chem. **A171,** 83 (1934).
(106) Hüttig, Radler, and Kittel: Z. Elektrochem. **38,** 442 (1932).
(107) Hüttig, Rosenkranz, Steiner, and Kittel: Z. anorg. allgem. Chem. **217,** 22 (1934).
(108) Hüttig and Zeidler: Kolloid-Z. **75,** 170 (1936).
(109) Hüttig, Zinker, and Kittel: Z. Elektrochem. **40,** 306 (1934).
(110) Hüttig, Tschakert, and Kittel: Z. anorg. allgem. Chem. **223,** 241 (1935).

(111) *International Critical Tables*, Vol. VI, p. 354. McGraw-Hill Book Company, Inc., New York (1929).
(112) Reference 111, p. 359.
(113) Reference 111, p. 360.
(114) Reference 111, p. 378.
(115) Reference 111, p. 405.
(116) Iskenderian: Phys. Rev. **51,** 1092 (1937).
(117) Jahr: Elektrochem. Z. **5,** 180 (1891).
(118) Jennison: J. Bact. **33,** 15 (1937).
(119) Juza and Biltz: Z. anorg. allgem. Chem. **205,** 284 (1932).
(120) Juza and Langheim: Naturwissenschaften **25,** 522 (1937).
(121) Juza and Langheim: Z. Elektrochem. **45,** 689 (1939).
(122) Juza, Langheim, and Hahn: Angew. Chem. **51,** 354 (1938).
(123) Kalckar and Teller: Proc. Roy. Soc. (London) **A150,** 520 (1935).
(124) Kaneko: J. Chem. Soc. Ind. Japan **34,** Suppl. binding, 133 (1931).
(125) Kitagawa: Rev. Phys. Chem. Japan **11,** 71 (1937).
(126) Kittel: Z. anorg. allgem. Chem. **222,** 1 (1935).
(127) Kittel: Z. anorg. allgem. Chem. **221,** 49 (1934).
(128) Kittel: Z. physik. Chem. **A178,** 81 (1936).
(129) Kittel and Hüttig: Z. anorg. allgem. Chem. **219,** 256 (1934).
(130) Kittel and Hüttig: Z. anorg. allgem. Chem. **217,** 193 (1934).
(131) Kittel, Hüttig, and Herrmann: Z. anorg. allgem. Chem. **212,** 209 (1933).
(132) Klemm: *Magnetochemie.* Akademische Verlagsgesellschaft, Leipzig (1936).
(133) Klemm: Z. anorg. allgem. Chem. **244,** 377 (1940).
(134) Klemm: Z. anorg. allgem. Chem. **246,** 347 (1941).
(135) Klemm: Z. anorg. allgem. Chem. **250,** 223 (1942).
(136) Klemm and Hass: Z. anorg. allgem. Chem. **219,** 82 (1934).
(137) Klemm and Schüth: Z. anorg. allgem. Chem. **203,** 104 (1931).
(138) Klemm and Schüth: Z. anorg. allgem. Chem. **210,** 33 (1933).
(139) Körber, Wiemer, and Fischer: Arch. Eisenhüttenw. **17,** 43 (1943).
(140) Kornfeld: Naturwissenschaften **16,** 1029 (1928); Z. physik. Chem. **B4,** 37 (1929).
(141) Kuhn: Freudenberg's *Stereochemie*, p. 917. F. Deuticke, Leipzig (1933).
(142) Lipin: J. Applied Chem. (U.S.S.R.) **16,** 258 (1943).
(143) Loeb: Am. Chem. J. **13,** 145 (1891).
(144) Marian: Ann. phys. **7,** 459 (1937).
(145) Mason: Trans. Am. Electrochem. Soc. **56,** 45 (1929).
(146) Mehmed and Haraldsen: Z. anorg. allgem. Chem. **235,** 193 (1938).
(147) Welo and Baudisch: Chem. Rev. **15,** 45 (1934).
(148) Welo and Baudisch: Phil. Mag. **3,** 396 (1927).
(149) Merck and Wedekind: Z. anorg. allgem. Chem. **186,** 49 (1930).
(150) Merck and Wedekind: Z. anorg. allgem. Chem. **192,** 113 (1930).
(151) Meyer: *International Critical Tables*, Vol. VI, p. 360. McGraw-Hill Book Company, Inc., New York (1929).
(152) Reference 151, p. 359.
(153) Reference 151, p. 357.
(154) Reference 151, p. 356.
(155) Meyer and Hüttig: Z. Elektrochem. **41,** 429 (1935).
(156) Michaelis and Granick: J. Gen. Physiol. **25,** 325 (1941).
(157) Michaelis and Granick: J. Biol. Chem. **148,** 463 (1943).
(158) Michel and Benard: Bull. soc. chim. **10,** 315 (1943).
(159) Michel and Gallissot: Compt. rend. **208,** 434 (1939).
(160) Winkel and Haul: Z. Elektrochem. **44,** 420 (1937).
(161) Montgomery and Ross: Phys. Rev. **43,** 358 (1933).
(162) Morris and Selwood: J. Am. Chem. Soc. **65,** 2245 (1943).

(163) Nichols: Am. J. Sci. **31**, 372 (1886).
(164) Noyes, Pitzer, and Dunn: J. Am. Chem. Soc. **57**, 1229 (1935).
(165) Parker and Armes: Trans. Roy. Soc. Canada **18**, Sec. III, 203 (1924).
(166) Post and Ham: J. Chem. Phys. **6**, 598 (1938).
(167) Ogawa: Tech. Rept. Kyushu Imp. Univ. **14**, 243 (1939).
(168) Olmer: Rev. mét. **38**, 129 (1941).
(169) Olmer: J. Phys. Chem. **46**, 405 (1942).
(170) Pantulu: Proc. Phys. Soc. (London) **47**, 197 (1935).
(171) Pascal: *International Critical Tables*, Vol. VI, p. 356. McGraw-Hill Book Company, Inc., New York (1929).
(172) Pauling: *Nature of the Chemical Bond.* Cornell University Press, Ithaca, New York (1939; 2nd edition, 1940).
(173) Perakis and Capatos: Compt. rend. **196**, 611 (1933); **198**, 1905 (1934).
(174) Rankine: Proc. Phys. Soc. London **46**, 1, 391 (1934).
(175) Rao and Savithri: Proc. Indian Acad. Sci. **16A**, 207 (1942).
(176) Rathert: Z. Elektrochem. **20**, 145 (1914).
(177) Raychaudhuri: Indian J. Phys. **9**, 417 (1935).
(178) Remsen: Am. Chem. J. **3**, 157 (1881).
(179) Rosenberg and Yuza: Ber. ukrain. wiss. Forsch.-Inst. physik. Chem. **2**, 36 (1929).
(180) Rowland and Bell: Phil. Mag. **26**, 105 (1888).
(181) Rüdorff and Stegeman: Z. anorg. allgem. Chem. **251**, 376 (1943).
(182) Sachse: Kolloid-Z. **58**, 18 (1932).
(183) Scheepers: Rev. universelle mines **19**, 121 (1943).
(184) Schüth and Klemm: Z. anorg. allgem. Chem. **220**, 193 (1934).
(185) Schwab and Agliardi: Ber. **73B**, 95 (1940).
(186) Selwood: *Magnetochemistry.* Interscience Publishers, Inc., New York (1943).
(187) Selwood and Nash: Trans. Am. Soc. Metals **35**, 609 (1945).
(188) Sieverts and Danz: Z. physik. Chem. **B38**, 61 (1937).
(189) Shchukarev: Z. physik. Chem. **113**, 441 (1924).
(190) Spingler and Reinhard: Z. physik. Chem. **A190**, 331 (1942).
(191) Steacie and Stevens: Can. J. Research **10**, 483 (1934).
(192) Stoner: *Magnetism and Matter.* Methuen and Company, Ltd., London (1934).
(193) Svensson: Ann. Physik. **18**, 299 (1933).
(194) Takatori: Science Repts. Tôhoku Imp. Univ. 1st Ser. **25**, 489 (1936).
(195) Taylor and Diamond: J. Am. Chem. Soc. **57**, 1251 (1935).
(196) Taylor and Diamond: J. Am. Chem. Soc. **55**, 2613 (1933).
(197) Theorell: J. Am. Chem. Soc. **63**, 1820 (1941).
(198) Theorell: Arkiv. Kemi, Mineral. Geol. **16A**, No. 3 (1943).
(199) Theorell and Agner: Arkiv Kemi, Mineral. Geol. **16A**, No. 7 (1943).
(200) Tilk and Klemm: Z. anorg. allgem. Chem. **240**, 355 (1939).
(201) Tjabbes: Proc. Acad. Sci. Amsterdam **35**, 693 (1932).
(202) Trew: Trans. Faraday Soc. **37**, 476 (1941).
(203) Turkevich: J. Chem. Phys. **12**, 345 (1944).
(204) Turkevich and Selwood: J. Am. Chem. Soc. **63**, 1077 (1941).
(205) Tyson and Wiley: J. Am. Chem. Soc. **66**, 1936 (1944).
(206) Van Itterbeck, Mariens, and Verpoorten: Nature **155**, 668 (1945).
(207) Van Vleck: *The Theory of Electric and Magnetic Susceptibilities.* Oxford University Press, Oxford (1932).
(208) Veil: Compt. rend. **180**, 932 (1925).
(209) Veil: Compt. rend. **182**, 1028 (1926).
(210) Vogt: "Magnetismus der metallischen Elemente," Ergeb. exakt. Naturw. **11** (1932).
(211) Wartmann: Phil. Mag. **30**, 266 (1847).
(212) Wedekind and Horst: Ber. **45**, 262 (1912); **48**, 105 (1915).

(213) Wedekind and Horst: *International Critical Tables*, Vol. VI, p. 359. McGraw-Hill Book Company, Inc., New York (1929).
(214) Weiss and Klemm: Z. anorg. allgem. Chem. **245**, 288 (1940).
(215) Wheeler: Phys. Rev. **41**, 331 (1932).
(216) Wigner: Z. physik. Chem. **B23**, 28 (1933).
(217) Williams: Phys. Rev. **27**, 484 (1926).
(218) Wolff: Am. Chem. J. **17**, 122 (1895).
(219) Woodman, Taylor, and Turkevich: J. Am. Chem. Soc. **62**, 1397 (1940).
(220) Zahn and Kramer: Physik. Ber. **22**, 991 (1941).
(221) Zimens and Hedvall: Svensk. Kem. Tid. **53**, 12 (1941).
(222) Zimens: Svensk Kem. Tid. **52**, 205 (1940).

THE REACTIONS OF UNSATURATED KETONES AND DERIVATIVES WITH AMINO COMPOUNDS

AMINO KETONES

NORMAN H. CROMWELL

Avery Laboratory of Chemistry, University of Nebraska, Lincoln, Nebraska

Received July 16, 1945

CONTENTS

I. Introduction 83
II. Reaction of amines with unsaturated ketones 84
  A. β-Amino ketones from vinyl ketones 84
  B. β-Amino-α,β-unsaturated ketones from acetylenic ketones 87
  C. Mechanism of addition 89
III. Reaction of amines with derivatives of unsaturated ketones 90
  A. Preparation of β-amino-α,β-unsaturated ketones 90
    1. Reaction of amines with 1,3-diketones 90
    2. Reaction of amines with β-substituted α,β-unsaturated ketones 93
    3. Mechanism of reaction and structure of products 95
      a. Structure of products 96
      b. The mechanism of the condensations 97
  B. Reaction of amines with bromo derivatives of unsaturated ketones 100
    1. Introduction 100
    2. Structure of the bromo amino ketones 109
    3. Reaction with primary amines. Ethylenimine ketones 113
    4. Mechanisms of the reactions 117
      a. With α-bromo-α,β-unsaturated ketones 117
      b. With α,β-dibromo ketones 119
    5. The preparation of mixed α,β-diamino ketones 120
IV. Reaction with carbonyl reagent type of amino compounds 121
V. Properties of amino ketones 124
  A. Saturated amino ketones 124
    1. Monoamino ketones 124
    2. Diamino ketones 128
  B. Unsaturated amino ketones 129
  C. Quinoline syntheses 130
    1. From vinyl ketones 130
    2. From β-diketones 132
VI. References 134

I. INTRODUCTION

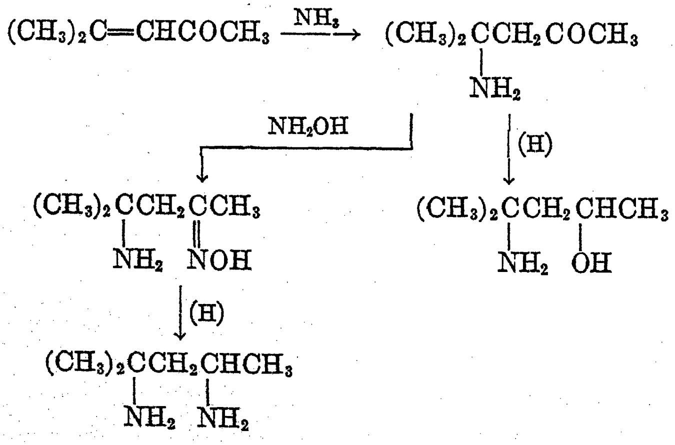
The reactions of vinyl ketones, acetylenic ketones, 1,3-diketones, and related derivatives with amino compounds yield as primary products various types of saturated and unsaturated amino ketones and heterocyclic compounds. The present review must be concerned mainly with these reactions, since the chemistry of some of the resulting types of amino ketones is not yet extensive. The study and development of such reactions has evolved several interesting theoretical considerations with regard to reaction mechanisms and molecular rearrangements. Moreover, preliminary studies of the resulting products have revealed interesting physical, chemical, and pharmacological properties in relation to their various structures. The amino ketones serve as starting materials or intermediates for the synthesis of various amino alcohols, diamines, heterocyclic nitrogen compounds, etc.

II. REACTION OF AMINES WITH UNSATURATED KETONES

A. β-AMINO KETONES FROM VINYL KETONES

The first reported reaction of an amino compound with a vinyl ketone (C=C—CO) was the reaction of ammonia with mesityl oxide, reported by Sokoloff and Latschinoff (109) in 1874. These early investigators had discovered, apparently simultaneously with Heintz (61), that acetone reacted with aqueous ammonia to form several condensation products, among which was the compound diacetone amine, to which the structure $(CH_3)_2C(NH_2)CH_2COCH_3$ was assigned. Sokoloff and Latschinoff showed that diacetone amine decomposed on heating to give ammonia and mesityl oxide. Furthermore, they demonstrated that mesityl oxide would add ammonia in aqueous solution to re-form the diacetone amine. Indeed, the modern method for preparing this important base involves similar conditions, although the product is now isolated as the more stable hydrated acid oxalate salt (96). Recently, Smith and Adkins (108) have shown that anhydrous ammonia adds readily to mesityl oxide at ordinary temperatures to give good yields of diacetone amine.

Evidence was eventually accumulated to show that diacetone amine contained a primary amino group and a carbonyl group, but no hydroxyl group. Heintz (62) reduced this β-amino ketone to the β-amino alcohol, using sodium amalgam in dilute hydrochloric acid solution. Harries (57) prepared the oxime and reduced it to 2-methyl-2,4-diaminopentane with sodium and alcohol.



$$\begin{tikzcd}
(CH_3)_2C{=}CHCOCH_3 \arrow[r, "NH_3"] & (CH_3)_2\underset{NH_2}{C}CH_2COCH_3 \arrow[dl, "NH_2OH"'] \arrow[d, "(H)"] \\
(CH_3)_2\underset{NH_2}{C}CH_2\underset{NOH}{C}CH_3 \arrow[d, "(H)"] & (CH_3)_2\underset{NH_2}{C}CH_2\underset{OH}{C}HCH_3 \\
(CH_3)_2\underset{NH_2}{C}CH_2\underset{NH_2}{C}HCH_3 &
\end{tikzcd}$$

Thus the general nature of the addition of amino compounds to α,β-unsaturated ketones has been known since the rather early beginnings of structural organic chemistry.

Following these early studies, mesityl oxide was shown to add in the same manner aliphatic amines in aqueous solution, such as methylamine (65), dimethylamine (65), and ethylamine (78). In recent years Jones and Kerner (71) added benzylamine, aniline, *m*-toluidine, and *p*-toluidine to mesityl oxide by heating the mixtures for several days to give about 25 per cent yields of the corresponding β-amino ketones.

In 1886 Beyer (13) developed a quinoline synthesis for which he postulated the intermediate formation of β-amino ketones from the addition of arylamines to α,β-unsaturated ketones. This method of quinoline synthesis is discussed in Section V,C,1. Tambor and Wildi (116) studied the reaction of ammonia and of many aromatic amines with benzalacetophenone. They found that this α,β-unsaturated ketone would add ammonia and primary aromatic amines, but not secondary aromatic nor mixed secondary aromatic–aliphatic bases. One molecule of the base was found to react with either one or two molecules of the unsaturated ketone (see table 1). In some cases it was necessary to add sodium hydroxide to the reaction mixtures to secure addition. Later, Georgi and Schwyzer (56) added piperidine, Stewart and Pollard (114) piperazine and morpholine, and Cromwell and Burch (34) tetrahydroisoquinoline to benzalacetophenone by heating the reagents together in hydrocarbon solvents or in alcohol. One mole each of morpholine, piperidine, and tetrahydroisoquinoline, respectively, reacted with 1 mole of benzalacetophenone, while 1 mole of piperazine reacted with 2 moles of the unsaturated ketone.

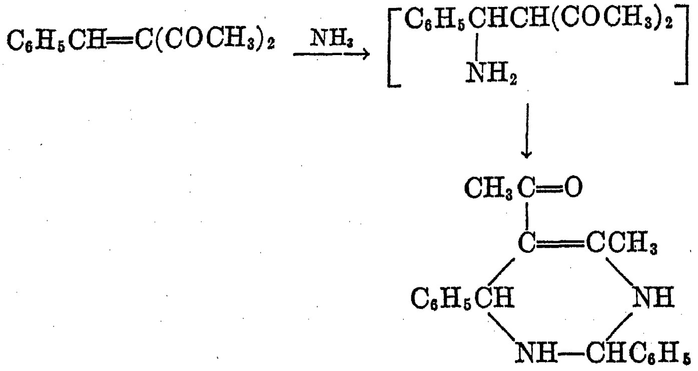
TABLE 1

β-Amino ketones from benzalacetophenone (116)

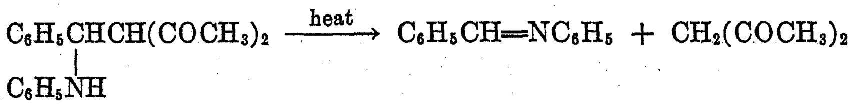
AMINE	ADDITION PRODUCT*	MELTING POINT
		°C.
Ammonia	$(C_6H_5COCH_2CHC_6H_5)_2NR$	163
Methylamine		167 (35)
o-Nitroaniline		243
p-Nitroaniline		252
m-Nitroaniline		240
p-Nitro-*o*-toluidine		203
α-Naphthylamine		180
Aniline	$(C_6H_5COCH_2CHC_6H_5)NHR$	175
p-Toluidine		172
β-Naphthylamine		200

* Only the products indicated could be isolated.

Benzalacetone has been shown to add methylamine in aqueous solution (79), aniline (90) and tetrahydroisoquinoline (34) in alcohol solution, and morpholine and piperidine in petroleum ether solutions (44) to give the expected β-amino ketones. Ruhemann and Watson (105), in an extensive investigation of the addition of organic bases to unsaturated ketones, found ammonia to form a heterocyclic product with α-acetylbenzalacetone and explained the reaction as follows:



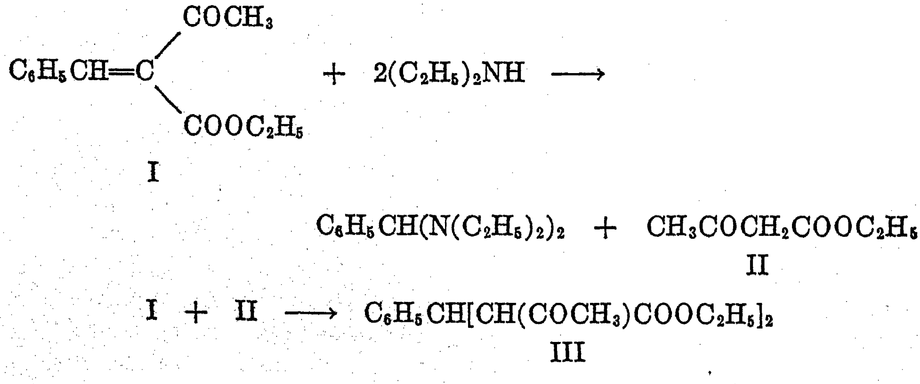
These investigators also were able to add aniline to this unsaturated system to obtain a β-aminoketone which on heating decomposed as indicated:



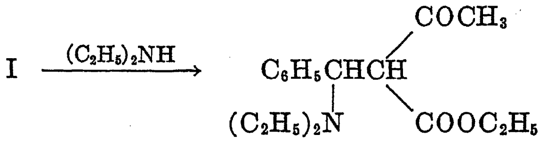
$$\underset{C_6H_5NH}{C_6H_5CHCH(COCH_3)_2} \xrightarrow{\text{heat}} C_6H_5CH{=}NC_6H_5 + CH_2(COCH_3)_2$$

In a later investigation (106) these workers were able to add *m*-toluidine, *m*-chloroaniline, *p*-chloroaniline, β-naphthylamine, and piperidine to obtain β-amino ketones, but were unable to add *o*-toluidine, α-naphthylamine, or tetrahydroquinoline to α-acetylbenzalacetone. Certain *o*-substituted benzenoid bases had previously been shown by Tambor and Wildi (116) to be relatively unreactive towards α,β-unsaturated carbonyl systems.

In 1898, Knoevenagel and Faber (72) had reported that ethyl benzalacetoacetate (I) in the presence of diethylamine gave ethyl benzylidinebisacetoacetate (III). They explained the reaction as follows:



Ruhemann and Watson (106), however, believed this reaction might best be explained by postulating the intermediate formation of a β-amino ketone (IV).



$$I \xrightarrow{(C_2H_5)_2NH} C_6H_5\underset{\substack{|\\ (C_2H_5)_2N}}{C}HCH\begin{matrix}\diagup COCH_3\\ \diagdown COOC_2H_5\end{matrix}$$

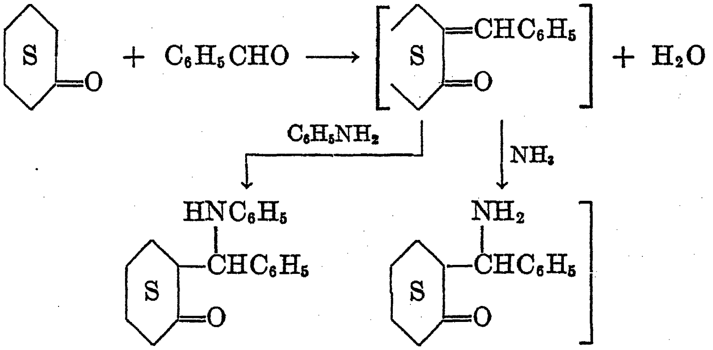
IV

$$IV \xrightarrow{H_2O} (C_2H_5)_2NH + C_6H_5CHO + II$$

$$IV + II \longrightarrow III + (C_2H_5)_2NH$$

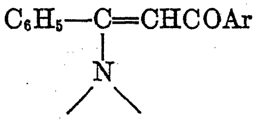
They offered as evidence for this mechanism the fact that piperidine, which adds to such systems, brings about the same reaction, while tetrahydroquinoline, which does not add readily, failed to give the reaction. Furthermore, it was pointed out that the presence of moisture was necessary for the formation of the final product.

An interesting series of reactions reported by Pirrone (97) seems to involve the intermediate formation of an α,β-unsaturated ketone, which then adds the amino compound in the usual way.



B. β-AMINO-α,β-UNSATURATED KETONES FROM ACETYLENIC KETONES

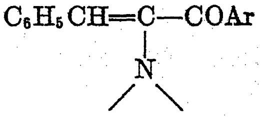
As a part of an investigation dealing with the preparation of acetylenic ketones, Watson (118) found that amines added readily to such compounds in a manner analogous to the addition of amines to olefinic ketones. Aniline was added to benzoylphenylacetylene to give a yellow crystalline product melting at 104°C., and *p*-methoxybenzoylphenylacetylene added piperidine to give a yellow product melting at 127°C. Watson was not able to decide between the structures A and B for these products.



$$C_6H_5{-}\underset{\substack{|\\ N\\ \diagup\ \diagdown}}{C}{=}CHCOAr$$

A

and



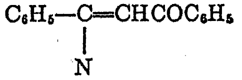
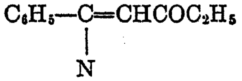
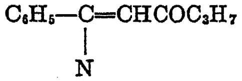
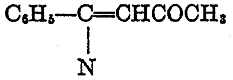
$$C_6H_5CH{=}\underset{\substack{|\\ N\\ \diagup\ \diagdown}}{C}{-}COAr$$

B

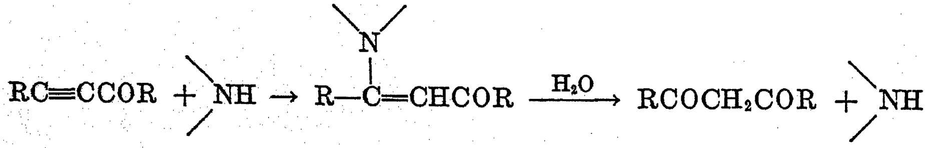
A few years later, in a more extensive investigation, André (3) added several primary and secondary aliphatic, heterocyclic, and aromatic amines to various acetylenic ketones, and proved these products to be β-amino-α,β-unsaturated ketones (see table 2).

TABLE 2

β-Amino-α,β-unsaturated ketones from acetylenic ketones (3)

AMINE	MELTING POINT
	 $C_6H_5-\underset{\substack{\mid\\ N}}{C}=CHCOC_6H_5$
	°C.
Benzylamine	100
Diethylamine	63
Piperidine	81, 99 (50)
N-Methylaniline	87
	 $C_6H_5-\underset{\substack{\mid\\ N}}{C}=CHCOC_2H_5$
Diethylamine	45
Cyclohexylamine	100
	 $C_6H_5-\underset{\substack{\mid\\ N}}{C}=CHCOC_3H_7$
Diethylamine	40
Cyclohexylamine	75
	 $C_6H_5-\underset{\substack{\mid\\ N}}{C}=CHCOCH_3$
Di-*n*-propylamine	47

These addition reactions were carried out in ether solutions either at room temperature or slightly above. Of all of the acetylenic ketones used, benzoylphenylacetylene seemed to be the most reactive. Since acid hydrolysis of these products gave β-diketones, this constituted a new synthesis of these compounds.



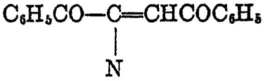
$$RC\equiv CCOR + \rangle NH \rightarrow R-\overset{\substack{\rangle N \langle\\ \mid}}{C}=CHCOR \xrightarrow{H_2O} RCOCH_2COR + \rangle NH$$

In more recent years Dupont (52) has added various amino compounds to diaroylacetylenes in toluene solution to give α-amino-α,β-diaroylethylenes (see table 3).

It is interesting to see that the acetylenic carbon atom in these compounds is sufficiently electrophilic to attract even the non-base pyrrole, a reaction which the author of this review has found to fail with various olefinic ketones.

TABLE 3

α-Amino-α,β-diaroylethylenes from diaroylacetylenes (52)

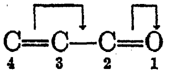
AMINE	MELTING POINT
	 $C_6H_5CO-\underset{\displaystyle N}{\underset{\vert}{C}}=CHCOC_6H_5$
	°C.
Ammonia	142
Dimethylamine	144
Piperidine	181
Aniline	121
β-Naphthylamine	143
Pyrrole	175

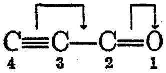
C. MECHANISM OF ADDITION

α,β-Unsaturated ketones

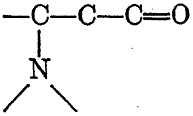
$$\overset{4}{C}=\overset{3}{C}-\overset{2}{C}=\overset{1}{O} \quad \text{and} \quad \overset{4}{C}\equiv\overset{3}{C}-\overset{2}{C}=\overset{1}{O}$$

in general undergo 1,2-, 3,4-, and 1,4-additions. The position favored depends on (*a*) the groups attached to the conjugated system, (*b*) the experimental conditions, and (*c*) the nature of the addenda. Factor (*b*) is of importance mainly in the consideration of the addition of such carbonyl reagents as hydroxylamine (see Section IV). It has been recognized for some time that the highly polar carbonyl group would be expected to confer electrophilic character on the olefinic carbon atom 4 by electromeric relay (99) in such conjugated systems.



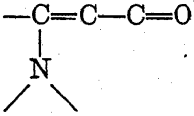


Since bases are also known to add to the carbonyl double bond, carbon atoms 2 and 4 must be expected to be in competition as electrophilic centers. The product of addition to such systems is not always determined by the relative rates of the two competing reactions. In fact, it seems probable in the case under consideration that the relative stability of the products resulting from the competing reactions is of major importance. That is,



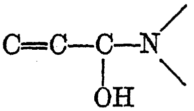
$-\underset{\displaystyle N}{\underset{\vert}{C}}-C-C=O$

and

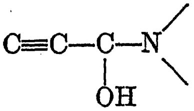


$-\underset{\displaystyle N}{\underset{\vert}{C}}=C-C=O$

are more stable and less susceptible to reversal than



and



especially when no hydrogen is available on the nitrogens for subsequent loss of water, as it is in oxime formation. Furthermore, other things being equal, resonance would be expected to favor the attack by the base at carbon atom 4 (see pages 468–70 of reference 20). Although the carbonyl group would be expected to activate the olefinic double bond and acetylenic triple bond, when conjugated with them, one does not expect the reverse to be true. The carbonyl group in such systems would be expected to be less reactive toward bases than when it occurs isolated.

The transition state for the addition of a base to a conjugated system has been described by Branch and Calvin (20). It seems logical that the driving force for such reactions is the attraction of the electrophilic carbon atom 4 for the unshared electrons of the amino nitrogen. It must be remembered, however, that the ability of amines to form C—N bonds, although related to, may not be directly proportional to the basic strengths of the amines. Although strong bases are strongly nucleophilic toward carbon, weaker bases retain a more than proportional activity toward carbon. The factor of stability of the product and the steric factor of ease of approach of the nitrogen atom to carbon atom 4 are of major importance (106).

One phase in the mechanism of the addition of bases to such conjugated systems that has not been extensively discussed as yet has to do with the mode of migration of the hydrogen that ultimately ends on carbon atom 3. One of the possible ways in which this may come about has been suggested by Cromwell and Cram (37), who postulate a six-membered chelate-ring structure as an intermediate.

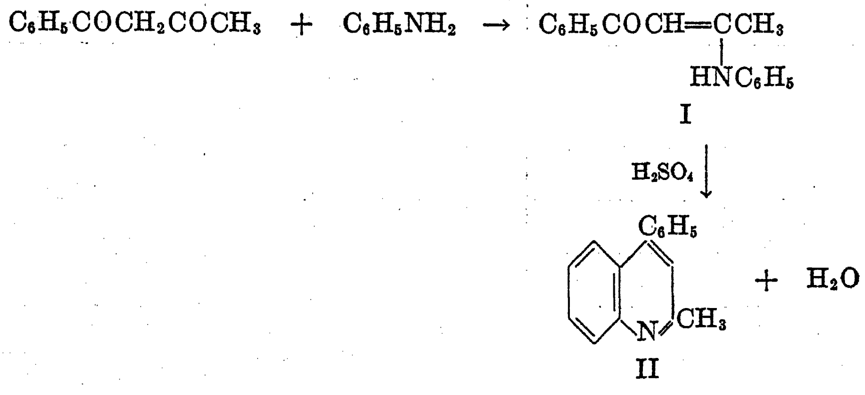
III. Reaction of Amines with Derivatives of Unsaturated Ketones

A. PREPARATION OF β-AMINO-α,β-UNSATURATED KETONES

1. Reaction of amines with 1,3-diketones

The first reaction of a β-diketone with an amino compound seems to have been carried out in 1885 by Fisher and Bülow (55). These workers showed that the newly discovered benzoylacetone reacted on being heated with concentrated ammonium hydroxide to give a base, $C_{10}H_{11}NO$, melting at 143°C., to which they did not assign a structure.

In his early studies with β-diketones, Beyer (14) developed an excellent method for preparing substituted quinolines, which he showed involved the intermediate formation of a β-amino-α,β-unsaturated ketone. When benzoylacetone was heated with aniline at 150°C., without solvent, or refluxed with it in glacial acetic acid, a basic product melting at 110°C. resulted. Since this substance was cyclized to the known γ-phenylquinaldine (II) in excellent yields in concentrated sulfuric acid, the structure was assigned as 1-phenyl-3-anilino-2-buten-1-one (I).



$$C_6H_5COCH_2COCH_3 + C_6H_5NH_2 \rightarrow C_6H_5COCH{=}C(NHC_6H_5)CH_3$$

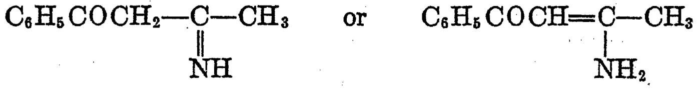
I

II

γ-Phenylquinaldine

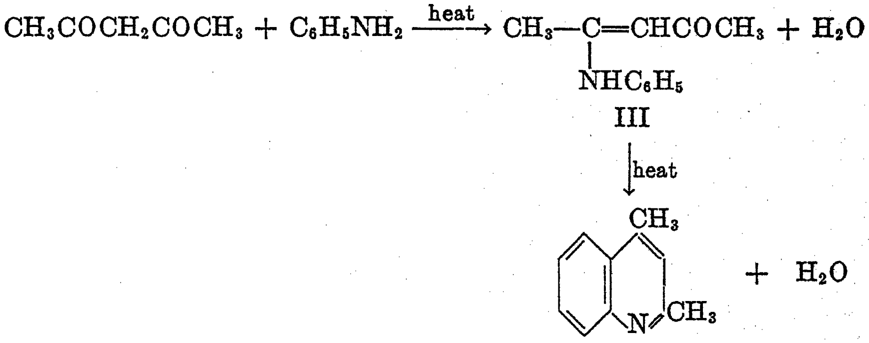
A detailed discussion of this and similar quinoline syntheses is given elsewhere in this review (section V,C,2).

Beyer and Claisen (16) reported in the same year that ammonia reacted with benzoylacetone to give a base melting at 143°C., the structure for which was written as



$$C_6H_5COCH_2{-}C({=}NH){-}CH_3 \quad \text{or} \quad C_6H_5COCH{=}C(NH_2){-}CH_3$$

by analogy with the proven aniline derivative. This was the base previously prepared by Fisher and Bülow (55). In the same year Combes (24) reported in the publication of his doctoral thesis on acetylacetone, that this β-diketone reacted with aniline to give a base boiling at 285–288°C. In the following year Combes (25) reported that this base could be cyclized by distillation or treatment with concentrated sulfuric acid to give 2,4-dimethylquinoline (IV) in excellent yields. Thus the structure of the base boiling at 285–288°C. and melting at 48°C. was written as indicated below (formula III):



$$CH_3COCH_2COCH_3 + C_6H_5NH_2 \xrightarrow{\text{heat}} CH_3{-}C(NHC_6H_5){=}CHCOCH_3 + H_2O$$

III

IV

TABLE 4

β-Amino-α,β-unsaturated ketones from β-diketones

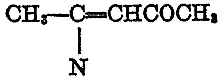
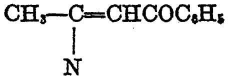
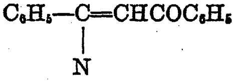
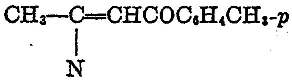
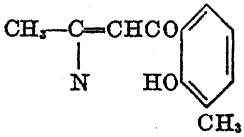
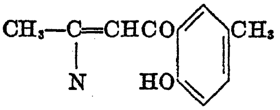
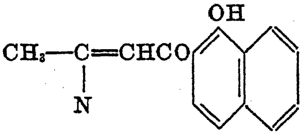
AMINE	MELTING POINT* OR BOILING POINT	REFERENCES
 $\mathrm{CH_3{-}\underset{\textstyle N}{\underset{\mid}{C}}{=}CHCOCH_3}$		
	°C.	
Ethylamine	215/760 mm.	(26, 103)
Diethylamine	156/24 mm.	(26)
Ethanolamine	73	(73)
Benzylamine	183/17 mm.	(104)
Aniline	48	(25)
 $\mathrm{CH_3{-}\underset{\textstyle N}{\underset{\mid}{C}}{=}CHCOC_6H_5}$		
Ammonia	143	(55, 16)
Methylamine	75	(15)
Diethylamine	71	(28)
Benzylamine	62	(33)
Cyclohexylamine	54	(33)
Aniline	110	(14)
Piperidine	98	(45)
Morpholine	146	(30)
 $\mathrm{C_6H_5{-}\underset{\textstyle N}{\underset{\mid}{C}}{=}CHCOC_6H_5}$		
Diethylamine	62	(28)
Benzylamine	101	(33)
Cyclohexylamine	78	(33)
Piperidine	No product	(45)
Morpholine	97	(29)
Aniline	103	(100)
 $\mathrm{CH_3{-}\underset{\textstyle N}{\underset{\mid}{C}}{=}CHCOC_6H_4CH_3\text{-}}p$		
Ammonia	93	(9)
Aniline	137	(9)
		
Ammonia	101	(123, 124)
Dimethylamine	110	(124)
Aniline	91	(124)

TABLE 4—*Concluded*

AMINE	MELTING POINT* OR BOILING POINT	REFERENCES
		
	°C.	
Ammonia	102	(124)
Aniline	106	(124)
		
Ammonia	154	(124)
Aniline	157	(124)

* Throughout these tables the boiling points are those numbers followed by a value for pressure, i.e., 156/24 mm.; all other values are melting points.

Since these early studies, various primary and secondary amines have been condensed with several β-diketones to give β-amino-α,β-unsaturated ketones, some of which are listed in table 4, while others are included in table 15.

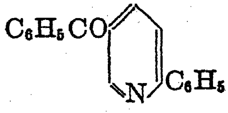
Wittig (123) reported in 1925 that 2-hydroxy-3-methylbenzoylacetone reacted with alcoholic ammonia to give two products to which he assigned the structures 3-amino-2,8-dimethylchromanone and 2-amino-2,8-dimethylchromanone, respectively. In a later more extensive investigation it was shown that such β-diketones react in the usual way with ammonia and amines to give the expected β-amino-α,β-unsaturated ketones (124) (see table 4).

2. *Reaction of amines with β-substituted α,β-unsaturated ketones*

Benary (11) has reported the preparation of a series of β-amino-α,β-unsaturated ketones by reacting several amine hydrochlorides in alcohol solution with the sodium salts of various β-hydroxy-α,β-unsaturated ketones of the type RCOCH=CHONa. Some of the products he prepared are given in table 5.

With hydroxymethyleneacetophenone and ammonium chloride the possible initial formation of the β-amino ketone (V) was apparently followed by a complex intermolecular condensation to give 2-phenyl-5-benzoylpyridine (VI), the only product isolated from this reaction.

$$C_6H_5COCH{=}CHONa + NH_4Cl \longrightarrow \underset{V}{[C_6H_5COCH{=}CHNH_2]} \longrightarrow$$



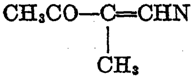
VI

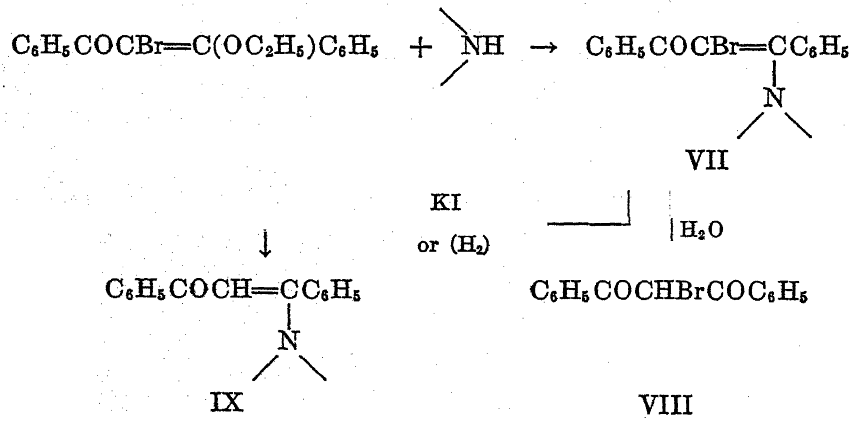
Several patents (53) have been granted for the preparation of β-amino-α,β-unsaturated ketones from β-halogenated α,β-unsaturated ketones ($RCOCH{=}CHCl$) and ammonia or amines in alcohol solution.

β-Alkoxy-α,β-unsaturated ketones ($RCOCH{=}C(OR)R$) react smoothly with amino compounds to form β-amino-α,β-unsaturated ketones. Dufraisse and Netter (51) reacted α-bromo-β-ethoxybenzalacetophenone with diethylamine, piperidine, and benzylamine to obtain the corresponding α-bromo-β-aminobenzalacetophenones (VII). The structures of these products were shown by their hydrolysis to bromodibenzoylmethane (VIII), and reduction to β-amino-α,β-unsaturated ketones (IX). The halogen in these products is stable to hydrolysis but labile to either catalytic reduction (37) or reduction with potassium iodide (51).

TABLE 5

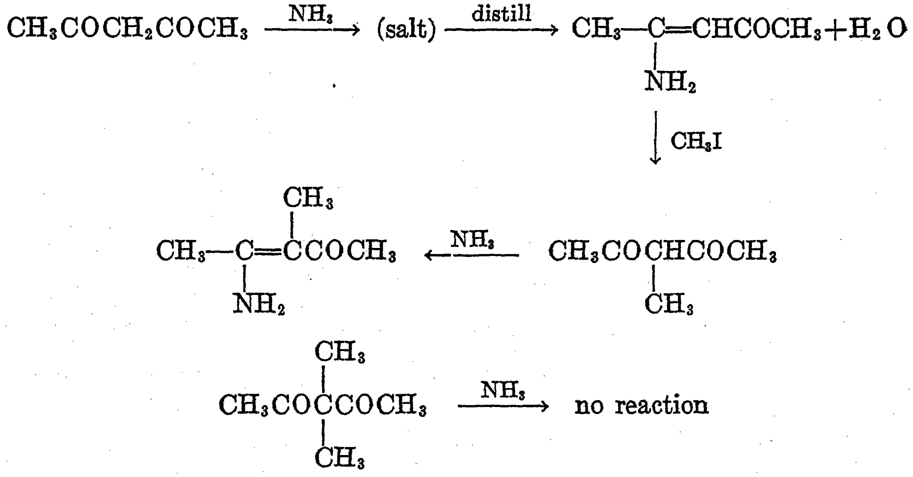
β-Amino ketones from β-hydroxy-α,β-unsaturated ketones (11)

AMINE	MELTING POINT OR BOILING POINT
	$CH_3COCH{=}CHN$
	°C.
Ammonia	96/14 mm.
Methylamine	93/17 mm.
Dimethylamine	118/19 mm.
Piperidine	162/16 mm.
	 $CH_3CO{-}\underset{\displaystyle CH_3}{\underset{\vert}{C}}{=}CHN$
Ammonia	112
Methylamine	58
Dimethylamine	55
	$C_6H_5COCH{=}CHN$
Piperidine	81



3. Mechanism of reaction and structure of products

During his rather extensive investigations of such compounds Combes (26) carried out the following series of reactions:



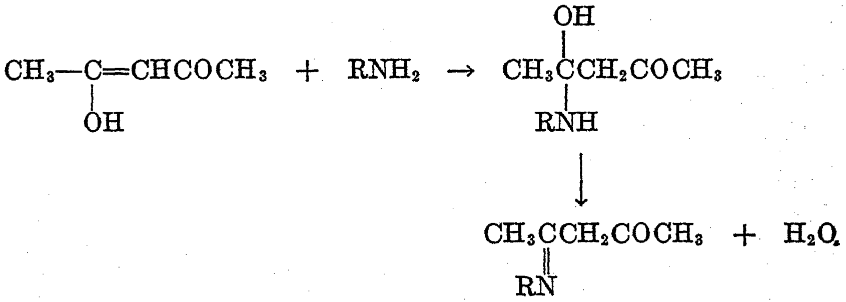
$$CH_3COCH_2COCH_3 \xrightarrow{NH_3} (\text{salt}) \xrightarrow{\text{distill}} CH_3\text{—}\underset{NH_2}{C}=CHCOCH_3+H_2O$$

$$\downarrow CH_3I$$

$$CH_3\text{—}\underset{NH_2}{C}=\overset{CH_3}{C}COCH_3 \xleftarrow{NH_3} CH_3CO\underset{CH_3}{C}HCOCH_3$$

$$CH_3CO\overset{CH_3}{\underset{CH_3}{C}}COCH_3 \xrightarrow{NH_3} \text{no reaction}$$

Since the α,α-dimethylacetylacetone gave no reaction with ammonia, Combes interpreted this as meaning that reaction will take place only when the grouping $(\text{—}\underset{OH}{C}=C\text{—})$ is available. Rügheimer (103, 104) also considered the reaction of amines with β-diketones to depend upon the enol form but preferred the imine structure to the vinylamine one for the product.

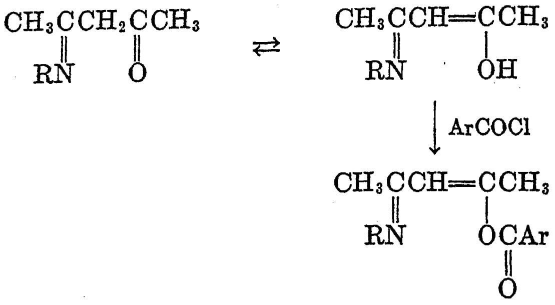


$$CH_3\text{—}\underset{OH}{C}=CHCOCH_3 + RNH_2 \rightarrow CH_3\overset{OH}{\underset{RNH}{C}}CH_2COCH_3$$

$$\downarrow$$

$$CH_3\underset{\overset{\|}{RN}}{C}CH_2COCH_3 + H_2O$$

Furthermore, he believed these amino or imino ketones quite capable of enolization. He was able to prepare benzoyl derivatives (X) which hydrolyzed readily, as do *O*-derivatives. His findings that these amino or imino ketones often give a color with ferric chloride may or may not be of importance, since such compounds are rapidly hydrolyzed in acid solution to the 1,3-diketones, which would be expected to give color with this reagent.

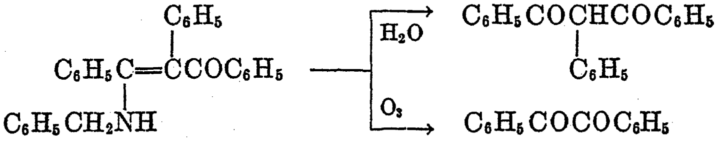


$$\underset{\substack{\| \\ \mathrm{RN}}}{\mathrm{CH_3C}}\mathrm{CH_2}\underset{\substack{\| \\ \mathrm{O}}}{\mathrm{C}}\mathrm{CH_3} \rightleftarrows \underset{\substack{\| \\ \mathrm{RN}}}{\mathrm{CH_3C}}\mathrm{CH{=}}\underset{\substack{| \\ \mathrm{OH}}}{\mathrm{C}}\mathrm{CH_3} \xrightarrow{\mathrm{ArCOCl}} \underset{\substack{\| \\ \mathrm{RN}}}{\mathrm{CH_3C}}\mathrm{CH{=}}\underset{\substack{| \\ \mathrm{OCAr} \\ \| \\ \mathrm{O}}}{\mathrm{C}}\mathrm{CH_3}$$

X

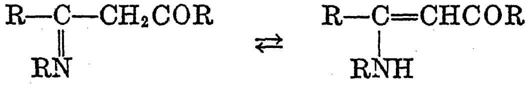
(a) Structure of products

Kohler and Blatt (75) studied the structure of β-benzylamino-α-phenylbenzalacetophenone and showed that hydrolysis resulted in the formation of dibenzoylphenylmethane, while ozonolysis gave benzil.



$$\underset{\substack{| \\ \mathrm{C_6H_5CH_2NH}}}{\mathrm{C_6H_5C}}{=}\overset{\substack{\mathrm{C_6H_5} \\ |}}{\mathrm{C}}\mathrm{COC_6H_5} \begin{cases} \xrightarrow{\mathrm{H_2O}} \mathrm{C_6H_5CO}\underset{\substack{| \\ \mathrm{C_6H_5}}}{\mathrm{C}}\mathrm{HCOC_6H_5} \\ \xrightarrow{\mathrm{O_3}} \mathrm{C_6H_5COCOC_6H_5} \end{cases}$$

This may be taken as evidence for the location of the β-benzylamino group, and the presence of the grouping $C_6H_5C{=}C(C_6H_5)COC_6H_5$. It must be admitted though, that such evidence is not particularly conclusive for the latter deduction because of the tautomeric relationship between the competitive structures and the possibility for rearrangement from one form to the other during reaction.



$$\mathrm{R{-}}\underset{\substack{\| \\ \mathrm{RN}}}{\mathrm{C}}\mathrm{{-}CH_2COR} \rightleftarrows \mathrm{R{-}}\underset{\substack{| \\ \mathrm{RNH}}}{\mathrm{C}}\mathrm{{=}CHCOR}$$

XI XII

On the basis of an extensive study of the exaltation of molecular dispersions of such substances, v. Auwers and Susemihl (7) have stated that these compounds always have the enamine (XII) not the imine (XI) structures (see table 6). Similar evidence dependent on the conjugated unsaturated nature of such substances has been reported in a study of color and constitution made by Cromwell and Johnson (42, 43) (see table 7).

In most cases the possibility for conjugation, with its accompanying stabilizing resonance energy, may be expected to favor the enamine structure (XII). However, if the nature of the R groups in XI ⇄ XII allows for a competing conjugated system in which the imine structure in XI may be involved, then resonance may in such cases stabilize the imine structure (XI).

TABLE 6

Exaltations of β-amino-α,β-unsaturated ketones (7)

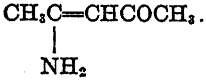
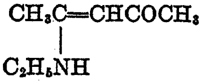
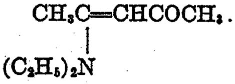
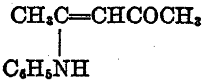
AMINO KETONES	$E(\Sigma_\beta - \Sigma_\alpha)$
	per cent
 $CH_3\underset{\displaystyle NH_2}{\underset{\vert}{C}}{=}CHCOCH_3$	185
 $CH_3\underset{\displaystyle C_2H_5NH}{\underset{\vert}{C}}{=}CHCOCH_3$	164
 $CH_3\underset{\displaystyle (C_2H_5)_2N}{\underset{\vert}{C}}{=}CHCOCH_3$	185
 $CH_3\underset{\displaystyle C_6H_5NH}{\underset{\vert}{C}}{=}CHCOCH_3$	198

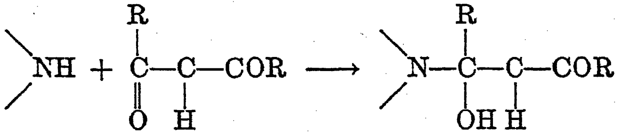
TABLE 7

Absorption spectra of β-amino-α,β-unsaturated ketones (42)

BENZALACETOPHENONES	MAXIMUM ABSORPTION BAND IN RANGE 3100–7000 Å. IN ABSOLUTE ALCOHOL	
	λ	$\epsilon \times 10^{-3}$
	Å.	
β-Diethylamino	3500	18.50
β-Morpholino	3500	20.48
β-Benzylamino	3450	14.58

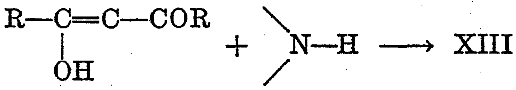
(b) The mechanism of the condensations

Very early in the study of the reactions of amines with 1,3-dicarbonyl compounds, it was shown that some intermediate compound was formed which then lost water to give the β-amino carbonyl substance (23, 26, 83). It is possible to write the formation of the intermediate compound in three different ways, one involving as the reactant the keto form, the other two the enol form.

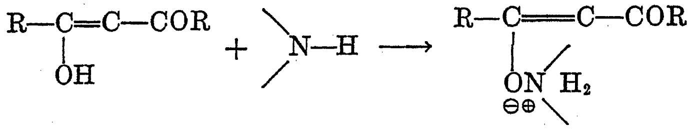
(1) 

$$\rangle NH + \underset{\underset{\displaystyle O}{\|}}{\overset{\overset{\displaystyle R}{|}}{C}}-\underset{\underset{\displaystyle H}{|}}{C}-COR \longrightarrow \rangle N-\underset{\underset{\displaystyle OH}{|}}{\overset{\overset{\displaystyle R}{|}}{C}}-\underset{\underset{\displaystyle H}{|}}{C}-COR$$

XIII

(2) 

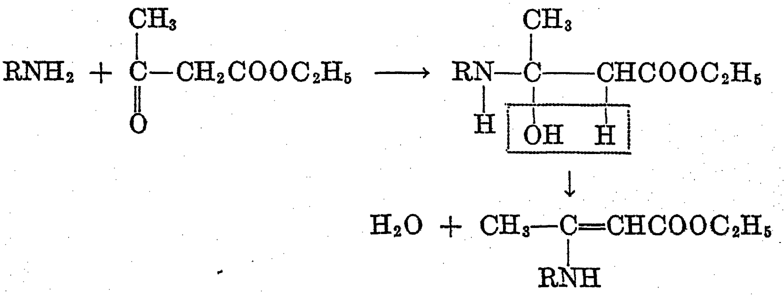
$$R-\underset{\underset{\displaystyle OH}{|}}{C}=C-COR + \rangle N-H \longrightarrow XIII$$

(3) 

XIV

It is of course well known that many of the 1,3-diketones exist mainly in the enol form (121). Furthermore, it is evident that when such highly enolized β-diketones as acetylacetone (26), dibenzoylmethane, and benzoylacetone are mixed with strongly basic amines, the immediate formation of a salt-like solid is experienced which must have the structure XIV, since these substances are often quite water soluble. Whether or not the formation of the salt (XIV) is important as an intermediate in the formation of the β-amino-α,β-unsaturated ketone (XII) is not answered by such evidence. It is quite possible that XIV must decompose to the starting materials, and the more important intermediate (XIII) then be formed by either course 1 or course 2. Also, even though these β-diketones exist mainly in the enol form, the energy required to convert from nearly all enol into nearly all keto form is so small that the keto form could well be the reactant, as shown in course 1 (see pages 286–87 of reference 20; also reference 27).

Recently Décombe (49) in his studies with the analogous β-keto esters and amines has chosen the keto form as the reactant in such condensations. His investigations indicated that the pure enolic form of acetoacetic ester did not react immediately with dry ammonia, while the equilibrium mixture in dry ether at 0°C. gave an intermediate crystalline compound which decomposed rapidly above 0°C. He wrote the mechanism for these reactions then, as:

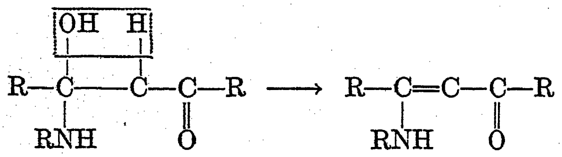


$$RNH_2 + \underset{\underset{O}{\|}}{\overset{\overset{CH_3}{|}}{C}}{-}CH_2COOC_2H_5 \longrightarrow \underset{\underset{H}{|}}{RN}{-}\underset{\underset{OH}{|}}{\overset{\overset{CH_3}{|}}{C}}{-}\underset{\underset{H}{|}}{C}HCOOC_2H_5$$

$$\downarrow$$

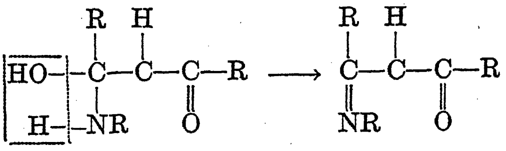
$$H_2O + CH_3{-}\underset{\underset{RNH}{|}}{C}{=}CHCOOC_2H_5$$

If the intermediate is XIII, water might split out according to either course 4 or course 5.

(4) 

$$R{-}\underset{\underset{RNH}{|}}{\overset{\overset{OH}{|}}{C}}{-}\overset{\overset{H}{|}}{C}{-}\underset{\underset{O}{\|}}{C}{-}R \longrightarrow R{-}\underset{\underset{RNH}{|}}{C}{=}C{-}\underset{\underset{O}{\|}}{C}{-}R$$

XIII XII

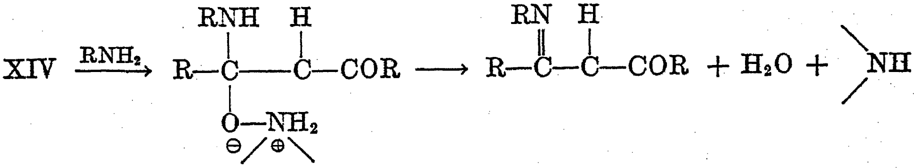
(5) 

$$\boxed{\begin{matrix}\text{HO}-\\ \text{H}-\end{matrix}}\underset{\underset{\text{NR}}{|}}{\overset{\overset{\text{R}}{|}}{\text{C}}}-\overset{\overset{\text{H}}{|}}{\text{C}}-\underset{\underset{\text{O}}{\|}}{\text{C}}-\text{R} \longrightarrow \underset{\underset{\text{NR}}{\|}}{\overset{\overset{\text{R}}{|}}{\text{C}}}-\overset{\overset{\text{H}}{|}}{\text{C}}-\underset{\underset{\text{O}}{\|}}{\text{C}}-\text{R}$$

XIII XI

For course 4 to be possible it is necessary that an α-hydrogen be available. Course 5 would be possible with no α-hydrogen available, but would take place only when hydrogen was available on nitrogen. It is certain that course 5 is impossible when secondary amines are used in these reactions. Moreover, it is to be recalled that Combes (26) was unable to condense ammonia with α,α-dimethylacetylacetone. This latter reaction might be expected if the mechanism of such condensations may be represented by course 1 followed by course 5. This is strong negative evidence against such a mechanism. Furthermore, it should be pointed out that the amino ketones resulting from the addition of amines to acetylenic ketones (see table 2) are identical with those resulting from the condensation of the same amines with analogous β-diketones (see table 4). However, whether XI or XII is first formed, the resulting product might be the more stable tautomer.

The fact that β-alkoxy-α,β-unsaturated ketones react readily with amines to give β-amino-α,β-unsaturated ketones may be taken as evidence that amines can add to such conjugated systems, but the isolation of the intermediate addition compounds has not been reported for such reactions. If the salt intermediate (XIV) is not important in such condensations, it might be expected that the preparation of certain β-amino-α,β-unsaturated ketones from strong amines and highly enolized ketones would be difficult. Cromwell and Witt (45) were not able to condense piperidine with dibenzoylmethane (intermediate salt formation was noted) to give the known β-piperidinobenzalacetophenone prepared by André (3) (see table 2) from benzoylphenylacetylene. In a private communication, Lutz (89) has reported the preparation of this amino ketone from β-ethoxybenzalacetophenone and piperidine. Salt formation might, however, be expected to favor enolization. Also, addition of the amine to the carbon-to-carbon double bond of the salt (XIV) can conceivably take place (103).



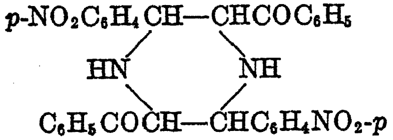
$$\text{XIV} \xrightarrow{\text{RNH}_2} \text{R}-\underset{\underset{\overset{\ominus}{\text{O}}-\overset{\oplus}{\text{NH}_2}\langle}{|}}{\overset{\overset{\text{RNH}}{|}}{\text{C}}}-\overset{\overset{\text{H}}{|}}{\text{C}}-\text{COR} \longrightarrow \text{R}-\overset{\overset{\text{RN}}{\|}}{\text{C}}-\overset{\overset{\text{H}}{|}}{\text{C}}-\text{COR} + \text{H}_2\text{O} + \rangle\text{NH}$$

Although no definite conclusion concerning the mechanism for these condensations of β-diketones with amines can yet be reached, the experimental results now available favor the following mechanism: 1,4-addition of the amine to the enol according to the scheme outlined for all α,β-unsaturated ketones in section II,C, followed by the loss of water from the intermediate (XIII) according to course 4.

B. REACTION OF AMINES WITH BROMO DERIVATIVES OF UNSATURATED KETONES

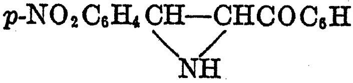
1. *Introduction*

The first reported reaction of a bromo derivative of an unsaturated ketone with an amino compound seems to have been the reaction of a dibromobenzalacetophenone with ammonia, carried out by Wieland in 1904 (122). The dibromide of *p*-nitrobenzalacetophenone reacted with alcoholic ammonia to give a colorless base melting at 141°C., for which Wieland wrote the structure



I

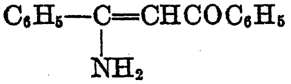
or



II

with preference expressed for formula I. No evidence was given to point to either structure. As will be brought out later (Section III,B,3), this actually was the first preparation of an ethylenimine of this type (II).

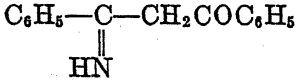
In the same year Ruhemann and Watson (106) reported the reaction of alcoholic ammonia with α,β-dibromobenzylacetophenone. They obtained a new, colorless base, m.p. 97°C., to which they assigned the structure:



$$C_6H_5-\underset{\substack{| \\ NH_2}}{C}=CHCOC_6H_5$$

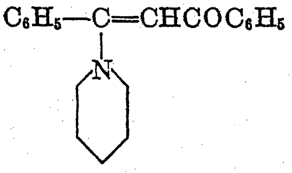
III

or



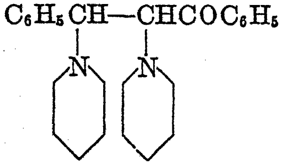
$$C_6H_5-\underset{\substack{\| \\ HN}}{C}-CH_2COC_6H_5$$

This product was shown to have a molecular weight corresponding to the indicated composition, and they pointed out that Wieland's product also probably contained only one benzalacetophenone residue. In a further extension of this work, Watson (118) obtained the same base, m.p. 97°C., from the reaction of alcoholic ammonia with the related α-bromobenzalacetophenone (he thought the structure was β-bromobenzalacetophenone). Furthermore, it was reported that piperidine reacted with such bromo derivatives. From the reaction of α-bromobenzalacetophenone with piperidine in alcohol solution two colored products were isolated which were described as follows:



IV

(red; m.p., 100°C.)

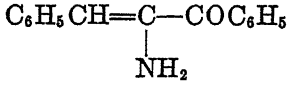


V

(yellow; m.p., 157°C.)

No evidence for these structures was presented.

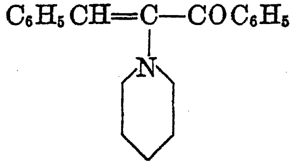
Much later, in 1927, Dufraisse and Moureu (50) began a series of investigations with bromo derivatives of unsaturated ketones with the apparent purpose of developing a method for preparing 1,2-diketones. The earlier work of Watson (118) was repeated, and on the basis of certain structure studies the formulas of Watson's products were changed to read:



$$C_6H_5CH{=}\underset{\displaystyle NH_2}{\underset{|}{C}}{-}COC_6H_5$$

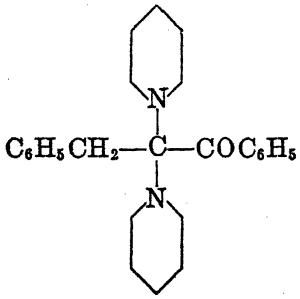
VI

(colorless; m.p., 95–97°C.)



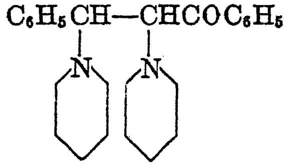
VII

(red; m.p., 100–101°C.)



VIII

or

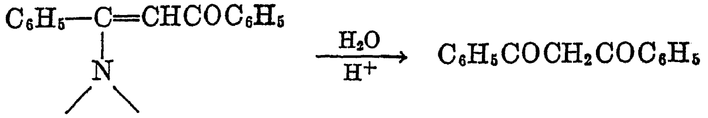


V

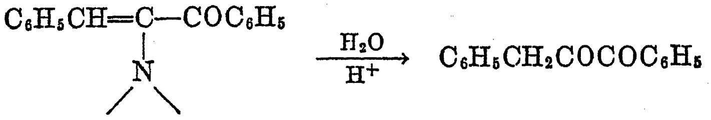
(yellow; m.p.,174°C.)

The red piperidino derivative (VII) was shown to hydrolyze readily with dilute acid to give 90 per cent yields of benzyl phenyl diketone. It was pointed out that André (3) had obtained β-piperidinobenzalacetophenone by adding piperidine to benzoylphenylacetylene. Such products of structure IX give only dibenzoylmethane when hydrolyzed with dilute acids. Furthermore, Dufraisse and Moureu found that acid hydrolysis of the aminobenzalacetophenone (structure III or VI) gave no dibenzoylmethane but did give small amounts of benzyl phenyl diketone and thus it was assigned structure VI. The evidence in this latter case was misleading, as will be brought out later (Section III,B,3).

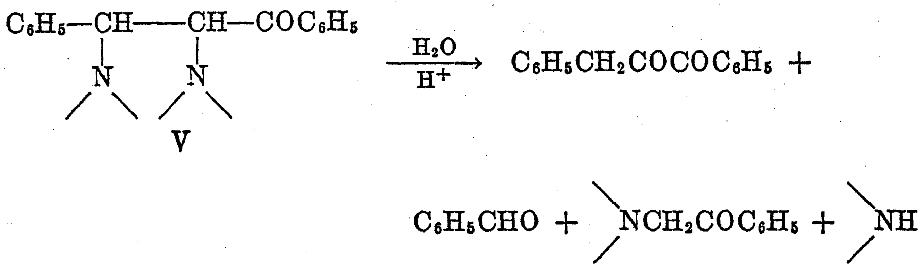
The structure of the dipiperidinobenzylacetophenone (structure V or VIII) was also studied by acid hydrolysis. Dufraisse and Moureu showed this substance to hydrolyze to give small amounts of benzyl phenyl diketone and larger amounts of benzaldehyde and ω-piperidinoacetophenone. Although this evidence definitely favors structure V for this substance, these investigators apparently preferred structure VIII, which should have given much larger amounts of benzyl phenyl diketone on such treatment.



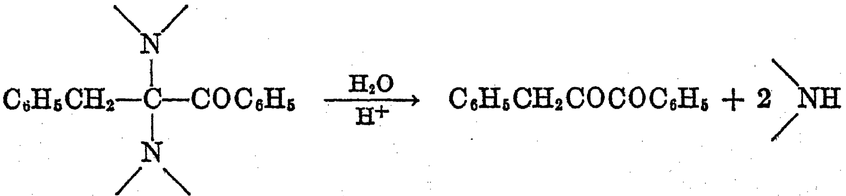
IX



VII

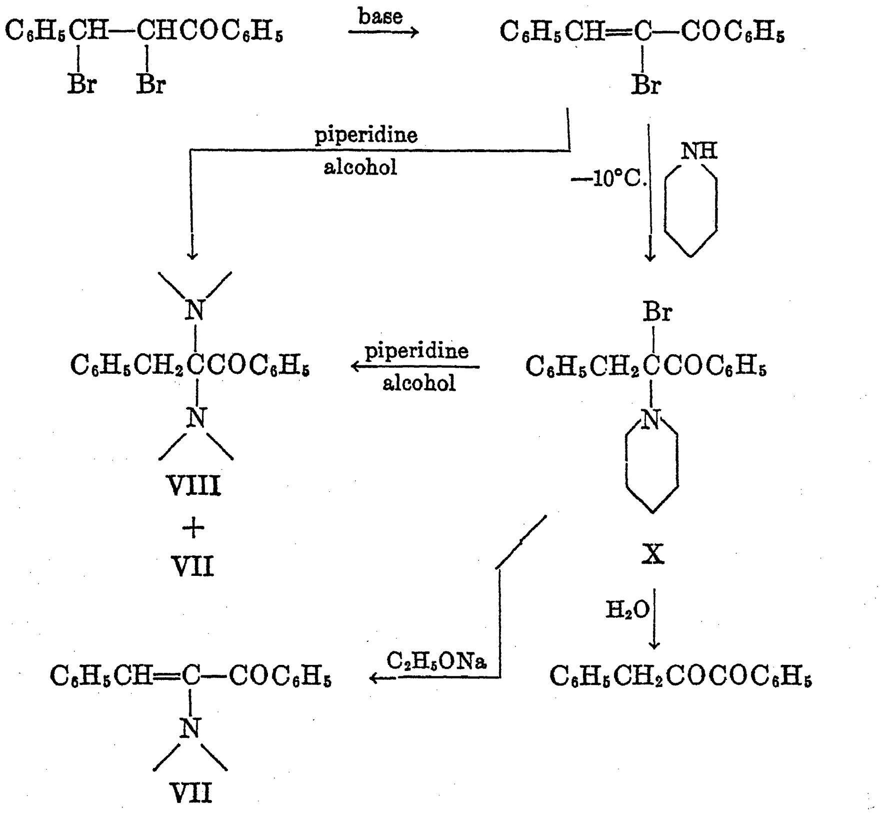


V



VIII

An important intermediate product of these reactions containing bromine and the piperidino group was isolated by Dufraisse and Moureu. When 1 mole of piperidine was added to a dry ether solution of 1 mole of α-bromobenzalacetophenone at −10°C., there resulted in 90 per cent yield a white product (X), m.p. 164°C., which gave a hydrobromide, m.p. 192°C. This substance was not very stable in air and reacted readily with sodium ethoxide to give 90 per cent yields of the red α-piperidinobenzalacetophenone (VII), m.p. 100–101°C., which had been obtained directly from either α-bromobenzalacetophenone or α,β-dibromobenzylacetophenone. With excess piperidine in alcohol solution X gave a mixture of the red monopiperidino compound (VII) and the yellow dipiperidino product (VIII). Furthermore, X was hydrolyzed by aqueous bases to give, along with other products, some benzyl phenyl diketone. On the basis of this evidence, Dufraisse and Moureu wrote reactions to explain all of these various changes as follows:

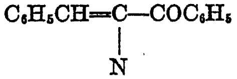
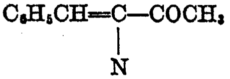
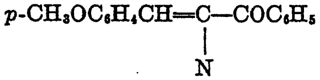


These investigators showed the action of piperidine on bromo derivatives of other unsaturated ketones to be analogous to the reactions described above, although the isolation of other intermediate bromo amino ketones of type X was not realized. According to the nature of the ketone, the reaction products were found to be made up of varying amounts of the monopiperidino and the dipiperidino products. No decision was reached with regard to the structures of the dipiperidino products, since they gave varying amounts of α-amino ketones and the 1,2-diketones on hydrolysis, depending on the nature of the starting bromo ketone. The various α-amino-α,β-unsaturated ketones and diamino ketones obtained from these studies are included in tables 8 and 9, respectively. Since the α-amino-α,β-unsaturated ketones are so readily hydrolyzed to 1,2-diketones, this constitutes an excellent method for preparing these substances (94).

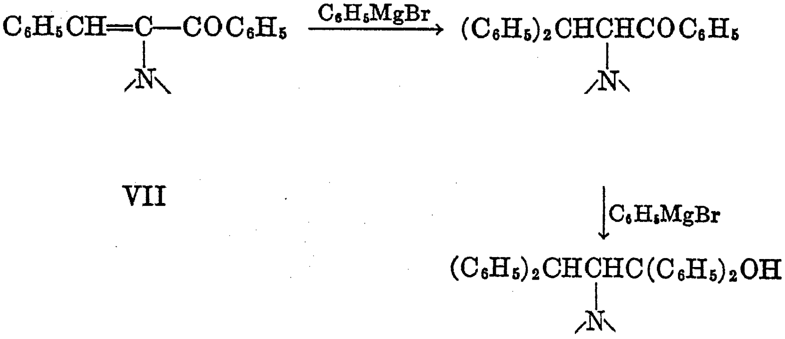
Kohler and Addinall (74) expressed the opinion that the structure VII for the α-piperidinobenzalacetophenone was incorrect. This view was revised in a subsequent communication (76), in which further evidence was offered for the structure (VII) of the α-piperidinobenzalacetophenone as described by Dufraisse and Moureu. This α-amino-α,β-unsaturated ketone was shown to react with the Grignard reagent in the normal way to give first a 1,4-addition product. The

TABLE 8

α-Amino-α,β-unsaturated ketones

	MELTING POINT		REFERENCES
 $C_6H_5CH{=}\underset{\substack{\vert\\N}}{C}{-}COC_6H_5$			
	°C.	*per cent*	
Dimethylamine	62		(112)
Diethylamine	53	89	(28)
Piperidine	102	81	(50)
Morpholine	96	75	(29)
Pyrrolidine	98	60	(32)
N-Methylbenzylamine	75	50	(45)
p-Methoxy-*N*-methylbenzylamine	82	74	(41)
 $C_6H_5CH{=}\underset{\substack{\vert\\N}}{C}{-}COCH_3$			
Piperidine	58	20	(45)
Morpholine	76	60	(30)
Tetrahydroisoquinoline	91	84	(37)
 $p\text{-}CH_3OC_6H_4CH{=}\underset{\substack{\vert\\N}}{C}{-}COC_6H_5$			
Piperidine	79	70	(50)

resulting saturated ketone then could be made to add a second mole of the Grignard reagent to give an amino carbinol.



$$C_6H_5CH{=}\underset{\substack{\vert\\ \diagup N \diagdown}}{C}{-}COC_6H_5 \xrightarrow{C_6H_5MgBr} (C_6H_5)_2CH\underset{\substack{\vert\\ \diagup N \diagdown}}{C}HCOC_6H_5$$

VII

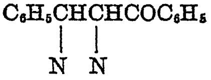
$$\downarrow C_6H_5MgBr$$

$$(C_6H_5)_2CH\underset{\substack{\vert\\ \diagup N \diagdown}}{C}HC(C_6H_5)_2OH$$

Reduction of α-piperidinobenzalacetophenone (VII) gave α-piperidinobenzylacetophenone (XI), which was also prepared from α-bromobenzylacetophenone. This saturated α-amino ketone reacted with phenylmagnesium bromide to give

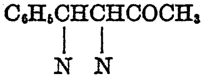
TABLE 9

α,β-Diamino ketones from α,β-dibromoketones



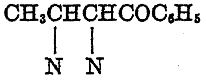
$C_6H_5\underset{\substack{|\\N}}{CH}\underset{\substack{|\\N}}{CH}COC_6H_5$

AMINE	MELTING POINT	YIELD	REFERENCES
	°C.	per cent	
Piperidine	174	32	(50)
Morpholine	175		
	156	70	(29)
Pyrrolidine	123	22	(32)
Tetrahydroisoquinoline	187	51	(38)
N-Methylbenzylamine	144		
	103	10	(45)



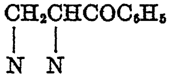
$C_6H_5\underset{\substack{|\\N}}{CH}\underset{\substack{|\\N}}{CH}COCH_3$

AMINE	MELTING POINT	YIELD	REFERENCES
Piperidine	122	35	(94, 45)
Morpholine	160	63	(30)
Tetrahydroisoquinoline	170	75	(37)
N-Methylbenzylamine	108	30	(45)
o-Hydroxy-*N*-methylbenzylamine	163	20	(41)
o-Methoxy-*N*-methylbenzylamine	114	80	(41)
p-Methoxy-*N*-methylbenzylamine	103	75	(41)



$CH_3\underset{\substack{|\\N}}{CH}\underset{\substack{|\\N}}{CH}COC_6H_5$

AMINE	MELTING POINT	YIELD	REFERENCES
Piperidine	114	65	(50)

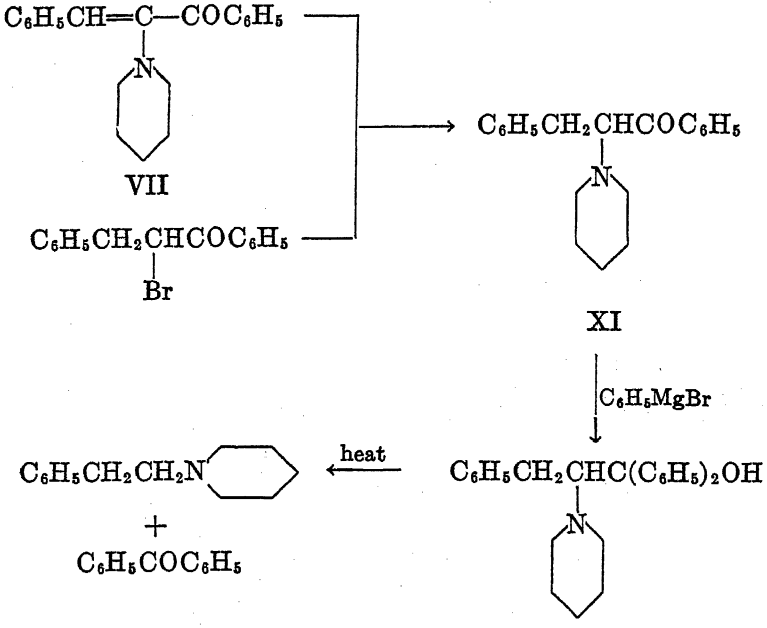


$\underset{\substack{|\\N}}{CH_2}\underset{\substack{|\\N}}{CH}COC_6H_5$

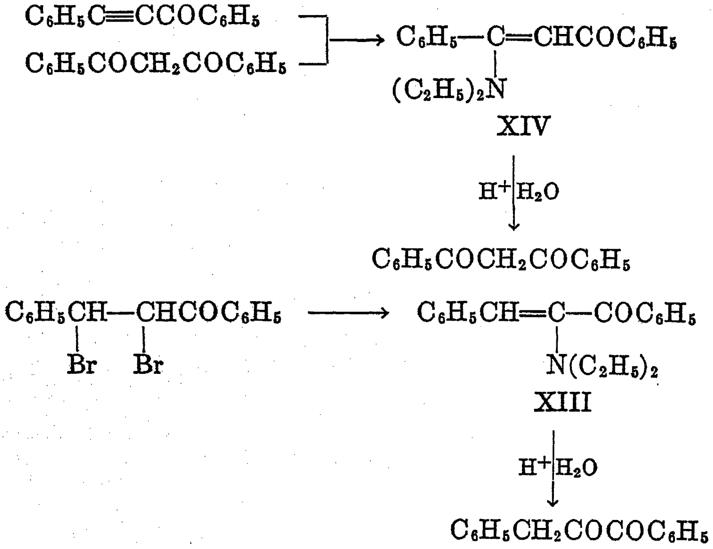
AMINE	MELTING POINT	YIELD	REFERENCES
Piperidine	73	90	(50)

an α-amino carbinol (XII), which on pyrolysis formed benzophenone and ω-piperidinoethylbenzene.

In 1940 a series of investigations was begun by Cromwell and his coworkers on the reactions of various amines with α,β-dibromo ketones and α-bromo-α,β-unsaturated ketones. In the first paper in the series (28), α,β-dibromobenzylacetophenone was shown to react with excess diethylamine in alcohol solution to give good yields of a red compound melting at 51–53°C., which was shown to be α-*N*-diethylaminobenzalacetophenone (XIII). Acid hydrolysis gave benzyl phenyl diketone.


VII XI XII

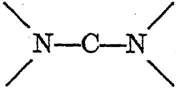
In this same communication the preparation of β-N-diethylaminobenzalacetophenone (XIV) by condensing dibenzoylmethane with diethylamine was also reported. This latter product was almost colorless, melted at 61–62°C., and was identical with the product previously obtained by André (3) by adding diethylamine to benzoylphenylacetylene. This definitely established the relationship between these two isomeric types of unsaturated amino ketones obtained by three different reactions.

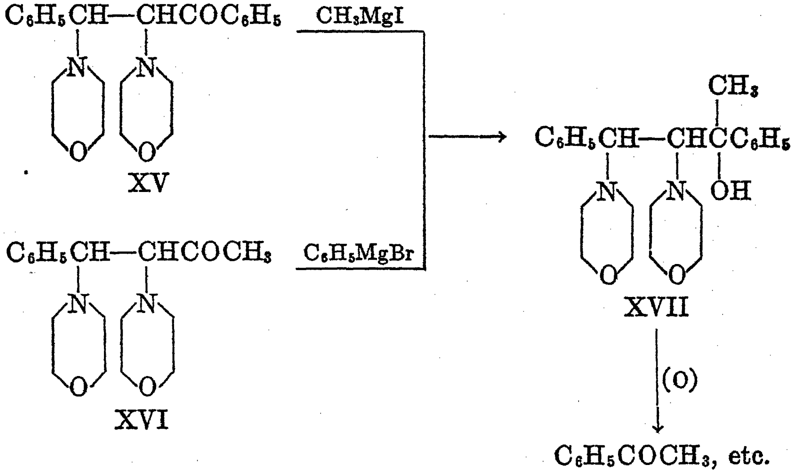


$$\left.\begin{array}{l} C_6H_5C\equiv CCOC_6H_5 \\ C_6H_5COCH_2COC_6H_5 \end{array}\right] \longrightarrow \underset{\text{XIV}}{C_6H_5\text{—}\underset{(C_2H_5)_2N}{C}=CHCOC_6H_5} \xrightarrow[H_2O]{H^+} C_6H_5COCH_2COC_6H_5$$

$$C_6H_5\underset{Br}{C}H\text{—}\underset{Br}{C}HCOC_6H_5 \longrightarrow \underset{\text{XIII}}{C_6H_5CH=\underset{N(C_2H_5)_2}{C}\text{—}COC_6H_5} \xrightarrow[H_2O]{H^+} C_6H_5CH_2COCOC_6H_5$$

It was not possible to obtain a bis-*N*-diethyl amino ketone similar to the dipiperidinoketone reported by Dufraisse in his studies. Subsequent investigations using morpholine, as Dufraisse had used piperidine, revealed the parallel nature of the behavior of these two heterocyclic secondary amines in these reactions (29, 30).

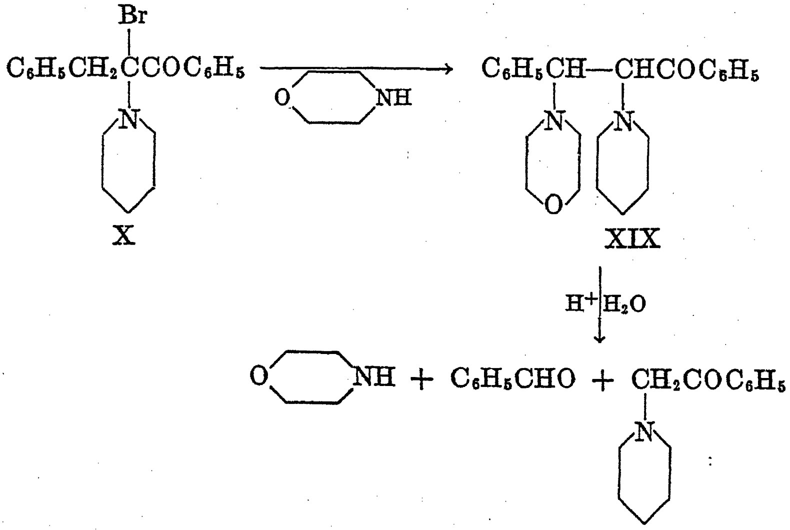
The α,β-dimorpholinobenzylacetophenone (XV) was obtained in a high (m.p. 175°C.) and a low (m.p. 154–156°C.) melting form, from α,β-dibromobenzylacetophenone. These were probably the two possible racemic mixtures (two dissimilar asymmetric carbon atoms are present). Both of these products hydrolyzed to give benzaldehyde, ω-morpholinoacetophenone, and only traces of benzyl phenyl diketone, indicating the structure to be that assigned, instead of the α,α-diamino ketone type of structure. α,β-Dibromobenzylacetone reacted with morpholine to give good yields of α,β-dimorpholinobenzylacetone (XVI). The structure of this product was shown to be analogous to that obtained in the benzylacetophenone series by application of the Grignard reaction as indicated. The products obtained from the two reactions were identical. This α,β-diamino carbinol (XVII) was stable to long boiling with dilute acid solutions, indicating the improbability of the presence of the structural unit . Furthermore, oxidation of the diamino carbinol (XVII) with dilute ammonium persulfate gave acetophenone, as expected.



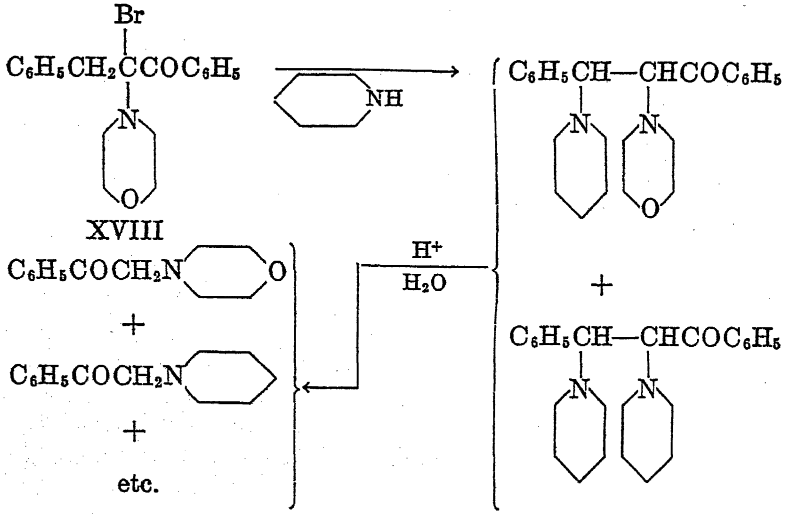
α-Bromobenzalacetophenone and the benzalacetone analogue both reacted with morpholine in dry ether solutions at low temperature to give (29, 30) excellent yields of bromo morpholino ketones, which showed the same properties as the analogous bromopiperidinobenzylacetophenone obtained by Dufraisse and Moureu and discussed previously in this review. Without further evidence, these structures were written by Cromwell as α,α-disubstituted ketones, analogous to the structure assigned by Dufraisse to his product.

In a following communication (31), the reactions of α-bromo-α-piperidinobenzylacetophenone (X) and the morpholino analogue (XVIII) with morpholine and piperidine, respectively, were reported. The first reaction was shown to give mainly α-piperidino-β-morpholinobenzylacetophenone (XIX), while the second reaction gave a mixed product which apparently contained several diaminobenzylacetophenones, as shown by acid hydrolysis and analysis. These reactions were written as follows:

(1)



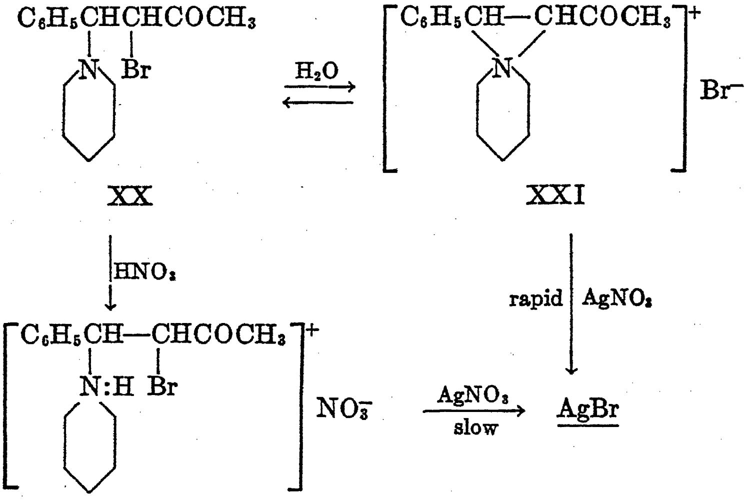
(2)



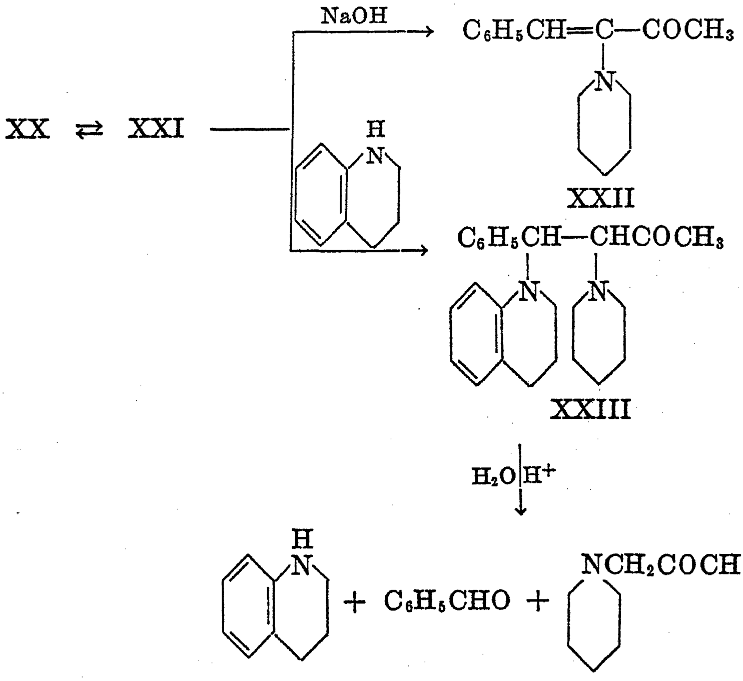
The reactions of series 1, especially, were offered as evidence for the location of the piperidino group on the α-carbon atom in the bromo amino ketones. These experiments did not explain how the second amino group arrived on the β-carbon. It was pointed out that it was not possible to add amines to either α-amino- or β-amino-α,β-unsaturated ketones to obtain α,β-diamino ketones.

2. Structure of the bromo amino ketones

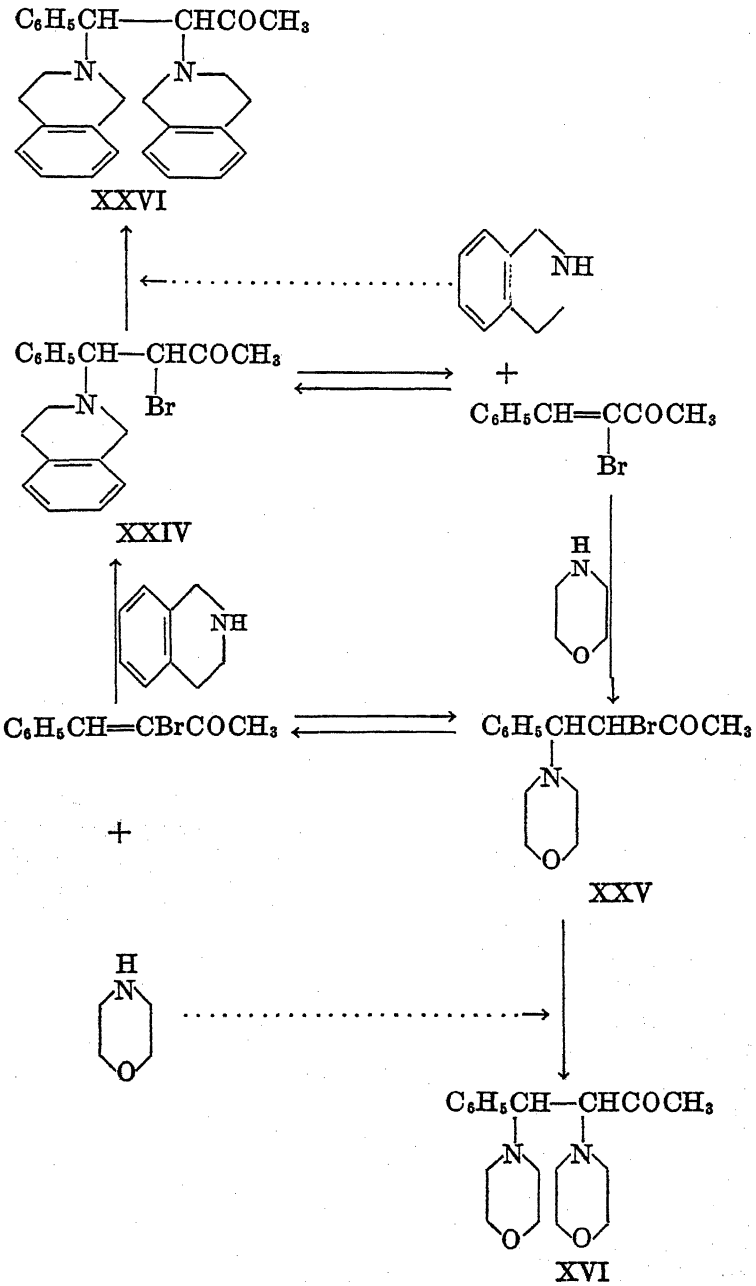
In 1943, after a more extensive investigation, evidence was presented to clarify these complex reactions. It was shown by Cromwell and Witt (45) that the intermediate bromo amino ketones were α-bromo-β-amino ketones (XX) which rearranged in neutral or basic solutions to form quaternary immonium salts (XXI) of the little-known ethylenimines. When XX was dissolved in dilute nitric acid and alcoholic silver nitrate added, only a very slow precipitation of silver bromide ensued. However, if XX was dissolved in aqueous alcohol, allowed to stand a few minutes, and then dilute nitric acid and silver nitrate added, an immediate precipitation of silver bromide took place. The initial presence of the acid involved the unshared electrons of the amino nitrogen and prevented the intramolecular attack on the α-carbon to displace the bromine as an ion and form XXI.



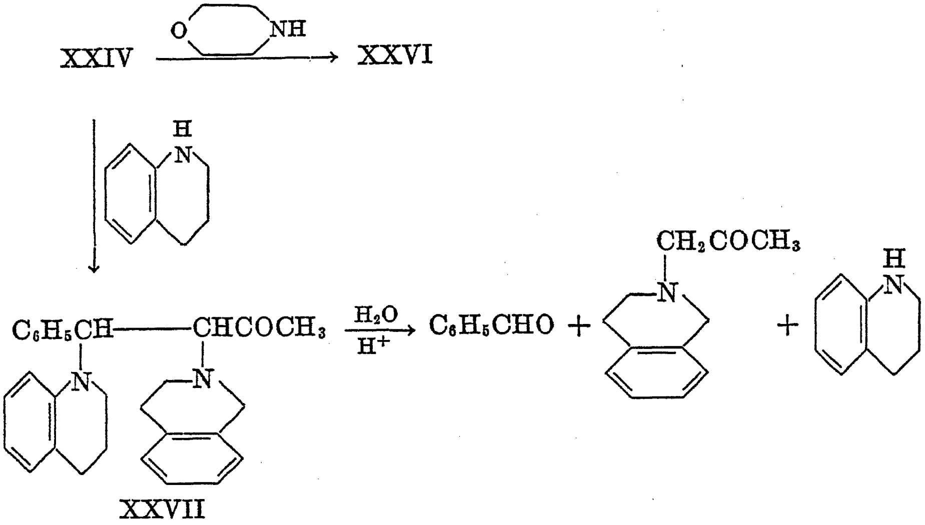
XX was shown to dissolve in water on long shaking to give a solution of XXI, which conducted the electric current and which reacted with sodium hydroxide to give α-piperidinobenzalacetone (XXII), and with tetrahydroquinoline to form α-piperidino-β-tetrahydroquinolinobenzylacetone (XXIII), the structure of which was shown by acid hydrolysis. These experiments served as further evidence for the α-bromo-β-amino ketone type of structure (XX) for these compounds.



In a parallel investigation, Cromwell and Cram (37) reported the preparation of many amino derivatives of benzalacetone and shed further light on the mechanisms of these reactions and the structures of the products. The nature of the important bromo amino ketones was studied further by an interesting series of experiments. If such compounds, XXIV and XXV, were allowed to stand in alcohol solution, a slow decomposition took place to re-form the amine and the α-bromo-α,β-unsaturated ketone. The released amine then reacted with the unchanged α-bromo-β-amino ketone, XXIV or XXV, respectively, to give the corresponding α,β-diaminoketone, XXVI and XVI, respectively. The filtrates from these solutions were then treated with some other amine to cause the released α-bromo-α,β-unsaturated ketone to react to form a different α,β-diamino ketone. These changes are shown in graphical form as follows:

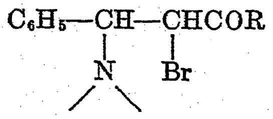


This series of experiments explained why it was possible to obtain complex products when bromo amino ketones were treated with certain amines, especially ones of the same or stronger basic strength compared with that of the amine used to prepare the bromo amino ketone. For example, the main product from the reaction of α-bromo-β-tetrahydroisoquinolinobenzylacetone (XXIV) with morpholine was α,β-ditetrahydroisoquinolinobenzylacetone (XXVI). The same bromo amino ketone reacted with a much weaker base, tetrahydroquinoline, to give excellent yields of the expected α-tetrahydroisoquinolino-β-tetrahydroquinolinobenzylacetone (XXVII), the structure of which was proven by acid hydrolysis.

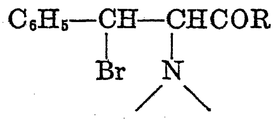


Moreover, the main product obtained by treating α-bromo-β-morpholinobenzylacetone with tetrahydroisoquinoline was also α,β-ditetrahydroisoquinolinobenzylacetone. When α-bromo-β-piperidinobenzylacetone was treated with tetrahydroisoquinoline (a weaker base than piperidine), a good yield of the expected α-piperidino-β-tetrahydroisoquinolinobenzylacetone resulted. On the other hand, when α-bromo-β-tetrahydroisoquinolinobenzylacetone was treated with piperidine (a stronger base than tetrahydroisoquinoline), the main product was again α,β-ditetrahydroisoquinolinobenzylacetone. Several other comparative reactions in the benzalacetone series were discussed by Cromwell and Cram (37), and in the benzalacetophenone series by Cromwell, Harris, and Cram (38).

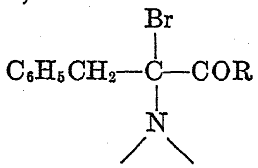
In an attempt to gain further evidence for the structure of these important bromo amino ketone intermediates, Cromwell and Cram (37) subjected α-bromo-β-piperidinobenzylacetophenone to mild catalytic reduction with the hope that the compound might be reduced to the known β-piperidinobenzylacetophenone. However, both the bromine atom and the piperidino group were removed, to give a 71 per cent yield of benzylacetophenone. This experiment favored the assigned, α-bromo-β-amino ketone structure (XXVIII).


$C_6H_5\text{—}\underset{\substack{|\\ N \\ /\ \backslash}}{CH}\text{—}\underset{\substack{|\\ Br}}{CH}COR$

XXVIII


$C_6H_5\text{—}\underset{\substack{|\\ Br}}{CH}\text{—}\underset{\substack{|\\ N \\ /\ \backslash}}{CH}COR$

XXIX


$C_6H_5CH_2\text{—}\overset{\substack{Br\\ |}}{\underset{\substack{|\\ N \\ /\ \backslash}}{C}}\text{—}COR$

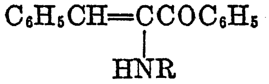
XXX

XXIX or XXX might be expected to reduce to the known α-piperidinobenzylacetophenone, which should be more stable under these conditions, while the product from XXVIII would be a β-amino ketone of a type which has been shown to be unstable to even mild catalytic reduction (44).

3. Reaction with primary amines. Ethylenimine ketones

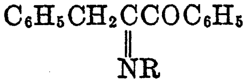
In Section III,B,1 ammonia, which may be looked upon as a primary amine, was mentioned as reacting with bromine derivatives of benzalacetophenone. Doubt was expressed as to the similarity in structure between these products and the products from the reactions of secondary amines with bromine derivatives of α,β-unsaturated ketones.

In 1943, Cromwell, Babson, and Harris (33) reported the reactions of two primary amines, benzylamine and cyclohexylamine, with α-bromobenzalacetophenone and α,β-dibromobenzylacetophenone as leading to the formation of ethylenimine ketones of the type shown in XXXIII:



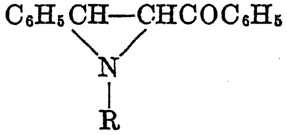
$C_6H_5CH{=}\underset{\substack{|\\ HNR}}{C}COC_6H_5$

XXXI



$C_6H_5CH_2\underset{\substack{\|\\ NR}}{C}COC_6H_5$

XXXII



XXXIII

Since the isomeric β-aminobenzalacetophenones had been prepared from dibenzoylmethane and by other means (3, 51) and found to possess entirely different properties (see Sections II,B and III,A,1) than these products resulting from bromine derivatives of benzalacetophenone, only structures XXXI, XXXII, and XXXIII needed to be considered.

It was shown (33) that products of structure XXXIII did not decolorize chloroform solutions of bromine as did the isomeric β-aminobenzalacetophenones. Also, the products having structure XXXIII were stable to catalytic hydrogenation with Raney nickel catalyst at room temperature, using hydrogen at 50 lb. per square inch.

The observation that all of the secondary amine reaction products of bromobenzalacetophenones possessed visible color, while the primary amine reaction products were colorless, led Cromwell and Johnson (42, 43) to carry out a study of color and constitution. As shown in table 10, the absorption spectra of compounds having the ethylenimine ketone structure (XXXIII) resemble that of the parent saturated ketone, benzylacetophenone.

As in the case of the reactions with secondary amines, it was possible (33) to add 1 mole of benzylamine to α-bromobenzalacetophenone to give α-bromo-β-benzylaminobenzylacetophenone (XXXIV), which possessed the general solubility properties of the previously discussed α-bromo-β-*sec*-amino ketones. This product reacted with various bases to produce the ethylenimine ketone (XXXV), which was also obtained directly from either α-bromobenzalacetophenone or α,β-dibromobenzylacetophenone.

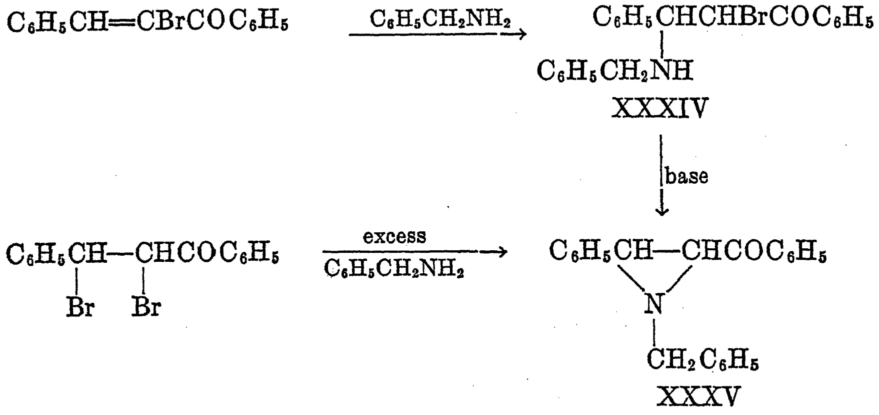


TABLE 10

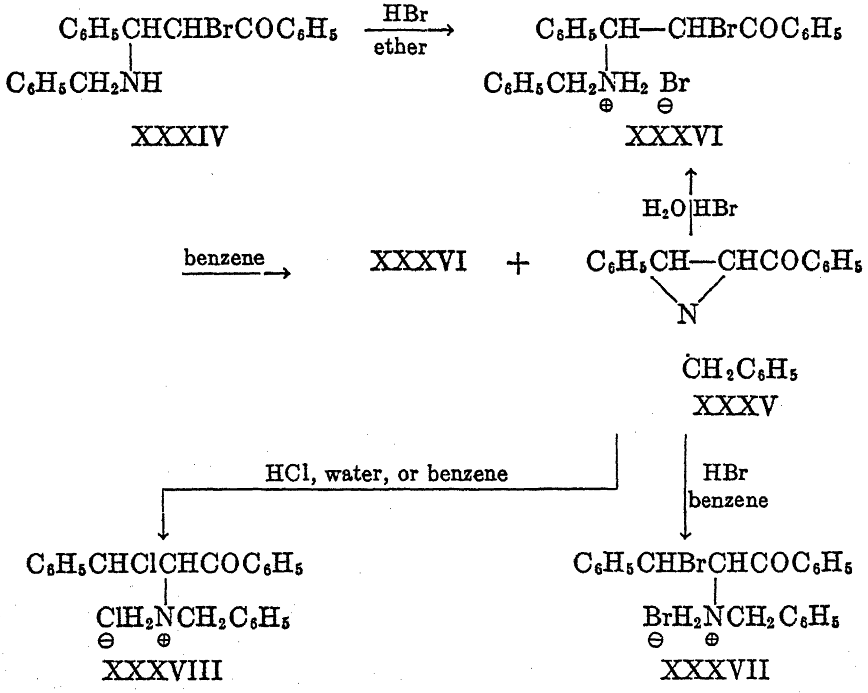
Absorption maxima of amino ketones (42)

KETONE	MELTING POINT	MAXIMUM ABSORPTION BAND IN RANGE FROM 3100-7000 Å.			
		Absolute alcohol		Dry benzene	
		λ	$\epsilon \times 10^{-3}$	λ	$\epsilon \times 10^{-3}$
	°C.	Å.		Å.	
Benzylacetophenone	72	3275	0.0418	3275	0.0331
2-Phenyl-3-benzoylethylenimine	101	3250	0.0430	3250	0.0465
1-Benzyl	108	3275	0.0623	3325	0.0686
1-Cyclohexyl	107	3300	0.0700	3350	0.0745
1-Methyl*	89			3325	0.0564
Benzalacetophenone	59	3350	2.040	3275	1.468
α-Diethylamino	54	4000	2.017	4000	2.820
α-Piperidino	102	3850	3.024		
α-*N*-Methylbenzylamino	75	4000	2.552		
β-Diethylamino	62	3500	18.50	3425	15.10
β-Benzylamino	101	3450	14.58		

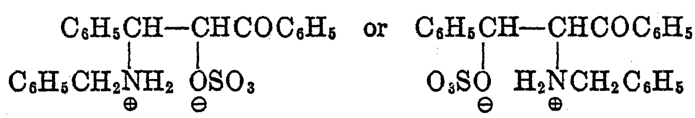
* See reference 35.

Ethylenimines have been prepared and studied by several investigators (47, 117, 119, 120). One of the most important reactions that such compounds undergo is their addition of 2 moles of hydrogen halide to form the hydrohalides of α-halo amino compounds. It was shown (33) that ethylenimine ketones of type XXXIII react with either dry or aqueous hydrogen halides to produce such products. Since the ethylenimine ring could open in two possible ways, it was necessary that some way be found to prove the structures of the resulting dihalides. In a recent paper, Cromwell and Caughlan (35) have found that such structures can be assigned on the basis of the relative rates of reactions of these hydrohalides of halo amino ketones with acidified potassium iodide solutions (see table 11).

These various dihalides were prepared in several ways (33, 35), as indicated in the following reactions:



These ethylenimine ketones also react (33) with dilute sulfuric acid to form an interesting type of aminosulfonic acid (XXXIX) (120):



$$\underset{\underset{\oplus}{C_6H_5CH_2NH_2}}{C_6H_5CH}-\underset{\underset{\ominus}{OSO_3}}{CHCOC_6H_5} \quad \text{or} \quad \underset{\underset{\ominus}{O_3SO}}{C_6H_5CH}-\underset{\underset{\oplus}{H_2NCH_2C_6H_5}}{CHCOC_6H_5}$$

XXXIX

In 1943, shortly after the paper by Cromwell, Babson, and Harris (33) had appeared, Algar, Hickey, and Sherry published an article (1) in which they described some interesting experiments involving the reactions of ammonia and methylamine with α,β-dibromobenzylacetophenone. These investigators did not isolate the intermediate products from these reactions but treated the crude products with alcoholic hydrogen chloride to obtain, as isolable products, the hydrochlorides of chloroaminobenzylacetophenones, apparently similar to those reported by Cromwell, Babson, and Harris (33).

In a recent reinvestigation of some of these reactions, Cromwell and Caughlan (35) have shown that methylamine behaves in the same manner as benzylamine and cyclohexylamine, giving 1-methyl-2-phenyl-3-benzoylethylenimine (XL), as shown graphically here:

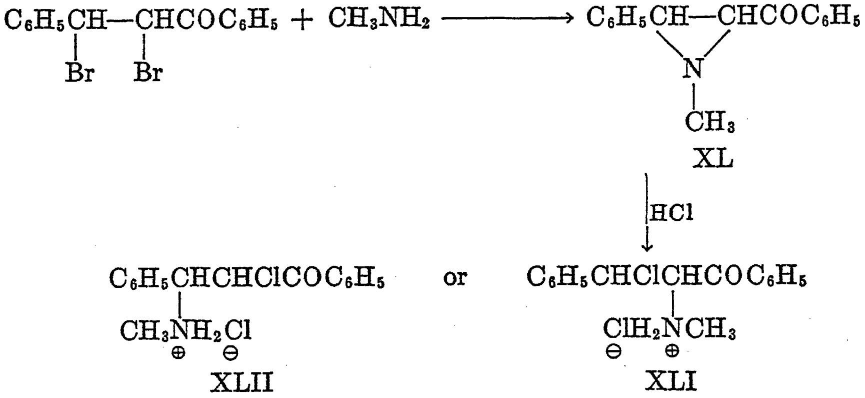
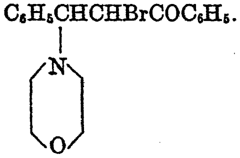
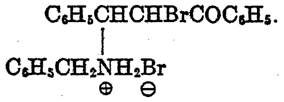
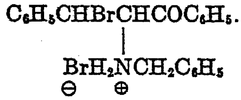
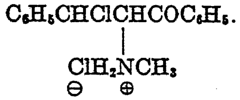
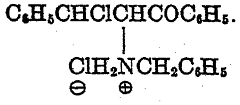


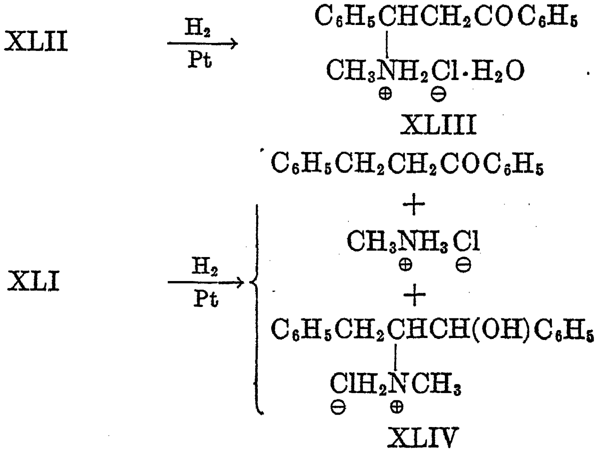
TABLE 11

Release of iodine from potassium iodide solutions by halogenated ketones (35)

HALOGENATED KETONES	PER CENT REACTING IN 30 MIN.
$C_6H_5CH_2CHBrCOC_6H_5$	27
 $C_6H_5CHCHBrCOC_6H_5$	79
 $\begin{array}{l}C_6H_5\underset{\vert}{C}HCHBrCOC_6H_5\\ C_6H_5CH_2\underset{\oplus}{N}H_2\underset{\ominus}{Br}\end{array}$	97
 $\begin{array}{l}C_6H_5CHBr\underset{\vert}{C}HCOC_6H_5\\ \underset{\ominus}{Br}H_2\underset{\oplus}{N}CH_2C_6H_5\end{array}$	0.87
 $\begin{array}{l}C_6H_5CHCl\underset{\vert}{C}HCOC_6H_5\\ \underset{\ominus}{Cl}H_2\underset{\oplus}{N}CH_3\end{array}$	0.93
 $\begin{array}{l}C_6H_5CHCl\underset{\vert}{C}HCOC_6H_5\\ \underset{\ominus}{Cl}H_2\underset{\oplus}{N}CH_2C_6H_5\end{array}$	0

The Irish workers wrote the structure for the dichloride as indicated by XLII rather than as XLI on the basis of the behavior of this product on catalytic hydrogenation. They reported that the dichloride could be reduced to give the hydrate of β-methylaminobenzylacetophenone hydrochloride (XLIII), m.p.

191°C. Cromwell and Caughlan (35) repeated this work and obtained a hydrochloride product melting in this range. The latter investigators, however, showed this product to contain methylamine hydrochloride and α,γ-diphenyl-β-methylaminopropanol hydrochloride (XLIV), and were not able to demonstrate the presence of XLIII.



$$\text{XLII} \xrightarrow[Pt]{H_2} \underset{CH_3\underset{\oplus}{N}H_2\underset{\ominus}{Cl}\cdot H_2O}{C_6H_5CHCH_2COC_6H_5}$$

XLIII

$$\text{XLI} \xrightarrow[Pt]{H_2} \begin{cases} C_6H_5CH_2CH_2COC_6H_5 \\ + \\ CH_3\underset{\oplus}{N}H_3\underset{\ominus}{Cl} \\ + \\ C_6H_5CH_2\underset{\underset{\ominus}{Cl}H_2\underset{\oplus}{N}CH_3}{C}HCH(OH)C_6H_5 \end{cases}$$

XLIV

Any of the structures XXXI, XXXII, and XXXIII might be expected to give varying amounts of 1,2-diketones when warmed with acids. It will be recalled (Section III,B,1) that α-amino-α,β-unsaturated ketones give almost quantitative yields of 1,2-diketones on such treatment. Thus the isolation of small amounts of 1,2-diketones from the hydrolysis of the reaction products of ammonia and primary amines with bromo derivatives of α,β-unsaturated ketones can not be taken as evidence for their structures.

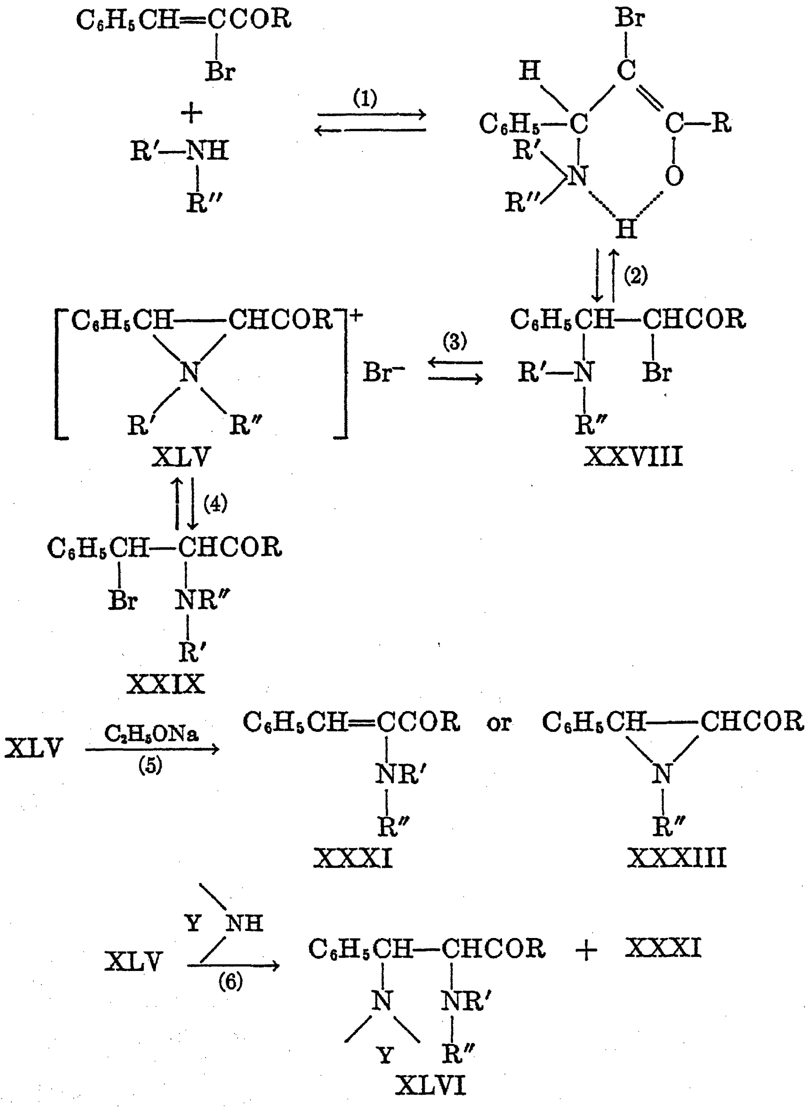
It seems evident that ammonia and primary amines react with α-bromo-α,β-unsaturated ketones and α,β-dibromoketones to give ethylenimine ketones of type XXXIII.

4. Mechanisms of the reactions

(a) With α-bromo-α,β-unsaturated ketones

The mechanism of the reaction of secondary amines with α-bromo-α,β-unsaturated ketones as assigned by Dufraisse (50) and elaborated by Cromwell (29) was incorrect. This first mechanism was based upon the assumption of the addition of the amine to the unsaturated ketone in such a manner as to form, as an intermediate, an α-bromo-α-amino ketone. Although such a structure for these intermediates could be expected to give the α-amino-α,β-unsaturated ketones, it could not be used to explain properly the formation of the α,β-diamino ketones, especially of the mixed type.

The evidence offered by the later investigations of Cromwell *et al.* (33, 35, 37, 38, 45) shows the structure of the addition products formed by the addition of amines to α-bromo-α,β-unsaturated ketones to be of the α-bromo-β-amino ketone type, as has been discussed previously (Section III,B,2). These active intermediates, which can often be isolated in almost quantitative yields, can then engage in various reactions in which the neighboring amino group participates during the removal or replacement of the halogen atom. Such a mechanism has been given by Cromwell and Cram (37).

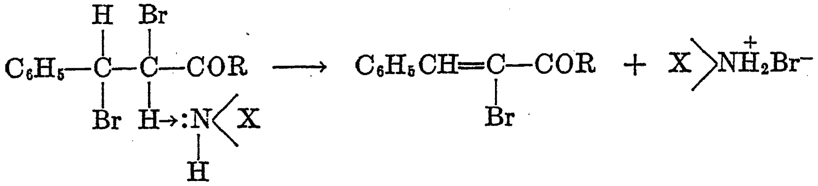


The addition of the amine to the α,β-unsaturated carbonyl system is usually quite rapid even at low temperatures and probably proceeds by some such mechanism as outlined in steps 1 and 2. Compounds of structure XXVIII are most readily obtained when the amine R′R″NH is of relatively high molecular weight and of considerable basic strength (morpholine, piperidine, pyrrolidine, tetrahydroisoquinoline, benzylamine, and *N*-methylbenzylamines). Furthermore, compounds XXVIII must be prepared in solvent media from which they will precipitate as rapidly as they are formed. In neutral or basic solutions XXVIII rearranges to form the important quaternary immonium salts, XLV, which under certain conditions may rearrange further to β-bromo-α-amino ketones (XXIX). That reactions 1 and 2 are reversible has been shown (37, 38) by allowing XXVIII to stand in solution to give "abnormal" products (see Section III,B,2).

Solutions of compounds of type XLV react almost quantitatively with a strong base such as sodium ethoxide to lose hydrogen bromide and give XXXI when R′R″NH is a secondary amine, and XXXIII when R′R″NH is ammonia or a primary amine. When solutions of XLV are treated with amines $\mathrm{Y}{>}\mathrm{NH}$ (primary or secondary) which are weaker bases than the amine R′R″NH (secondary amine) previously used to form XXVIII, then good yields of XLVI are to be expected, if no steric factor is involved. It has been reported (36) that when $\mathrm{Y}{>}\mathrm{NH}$ is an open-chain secondary amine (*N*-methylbenzylamine or dibenzylamine) the formation of XLVI is slowed because the approach of the amino nitrogen to the β-carbon atom in XLV is hindered to such an extent that larger amounts of XXXI are formed. The formation of XXXI is less dependent on the structure of the base $\mathrm{Y}{>}\mathrm{NH}$ and more dependent on its basic strength than is the formation of XLVI. In XLV the α-carbon–nitrogen bond should be the strongest, and thus the incoming amino group $\mathrm{Y}{>}\mathrm{N}{<}$ would be expected to take the β-position, especially when $\mathrm{Y}{>}\mathrm{NH}$ is a weaker base than R′R″NH. Ethylenimines (XXXIII) are to be expected from the treatment of XLV with amines $\mathrm{Y}{>}\mathrm{NH}$ when R′R″NH is a primary amine (33).

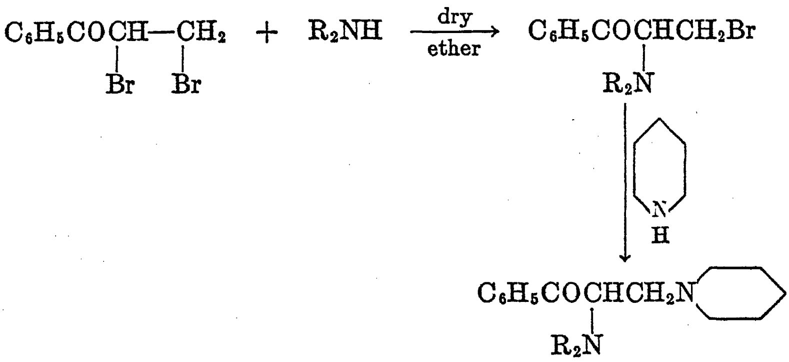
(b) With α,β-dibromo ketones

The dibromo ketones react with secondary amines to give mixtures of the α,β-diamino ketones and the α-amino-α,β-unsaturated ketones in varying proportions. In general, the best yields of the α,β-diamino ketones result when the amine used is of moderate strength (morpholine, tetrahydroisoquinoline, and methoxybenzylamines) (see table 9). With stronger amines (diethylamine and piperidine) the α-amino-α,β-unsaturated ketones are formed in larger amounts. The first step in the reactions of the α,β-dibromo ketones with amines in some cases is probably the loss of hydrogen bromide to form the α-bromo-α,β-unsaturated ketone, which then reacts further according to the mechanisms outlined above.



$$C_6H_5-\overset{H}{\underset{Br}{C}}-\overset{Br}{\underset{H\rightarrow :\underset{H}{N}\langle X}{C}}-COR \longrightarrow C_6H_5CH{=}\underset{Br}{C}-COR + X\rangle \overset{+}{N}H_2Br^-$$

An alternative mechanism, possibly parallel in some cases and exclusive in others, is one involving direct attack of the amine at the carbon atoms holding the bromine atoms. Davis (48) has reported an investigation in which the amine has been indicated as attacking the α-carbon atom first in such compounds. A series of mixed α,β-diamino ketones was prepared, the structures for which were reported without proof, as shown below:



(R = CH_3; C_2H_5; n-C_3H_7; n-C_4H_9; n-C_5H_{11})

The limitations of this method of preparing mixed α,β-diamino ketones from α,β-dibromo ketones of the type $C_6H_5COCHBrCHBrR$, where R is alkyl or aryl, have not been reported. The nature of R would be expected to influence the course of such reactions.

5. The preparation of mixed α,β-diamino ketones

A wide variety of mixed diamino ketones may be prepared starting with α-bromo-α,β-unsaturated ketones. From a knowledge of the mechanisms of these reactions it became possible to arrange the experimental conditions in such a manner as to allow for the maximum yields. In general, the α-bromo-β-amino ketones are prepared in dry ether solutions at low temperatures, isolated, and used immediately in the next step of the preparation. The formation of the mixed diamino ketone from the α-bromo-β-amino ketone is usually carried out in absolute alcohol, although better yields have been obtained in a few cases in dry ether solutions when the amine being used was of almost the same basic strength as that of the amine used to form the α-bromo-β-amino ketone.

The structures of the mixed α,β-diamino ketones have been proven for various typical types by hydrolysis, as shown in Sections III,B,1 and III,B,2. A different method for preparing certain mixed α,β-diamino ketones has been reported by Davis (48) and is discussed at the end of Section III,B,4b. In table 12 are listed the various mixed α,β-diamino ketones that have been prepared by the former method.

IV. REACTION WITH CARBONYL REAGENT TYPE OF AMINO COMPOUNDS

As has been shown in the preceding sections, ammonia and amines can usually be expected to react exclusively with the ethylenic linkage in α,β-unsaturated ketones. With amino compounds that are usually considered to be carbonyl reagents, α,β-unsaturated ketones show reactivity at both the ethylenic and the carbonyl linkages, as they do with other carbonyl reagents such as sodium bisulfite, the Grignard reagent, etc.

In acidic media α,β-unsaturated ketones react with carbonyl reagent types of amino compounds to give hydrazones, phenylhydrazones, semicarbazones, and oximes. In alkali media the reactions are more complex and products result from the addition at both the carbonyl and the ethylenic linkages. Furthermore, in alkali media, secondary products result from reactions involving active hydrogen in the primary addition products and also from oxidation–reduction reactions. In an excellent series of three review papers (4, 5, 6), v. Auwers and Muller have reviewed the earlier work on the reactions of hydroxylamine with benzalacetophenone and dibenzoylmethane, and with benzalacetone, benzoylacetone, and ethylideneacetone. Also, detailed directions are given for the preparation of the great variety of products possible from these reactions.

Blatt (18) has shown that, in contrast to hydroxylamine, methoxylamine adds only at the ethylenic bond in α,β-unsaturated ketones. Benzalacetophenone reacted to give β-methoxyaminobenzylacetophenone, m.p. 54–55°C. The hydrochloride of I was oxidized with hypochlorous acid to II, which was also obtained from dibenzoylmethane and from benzoylphenylacetylene.

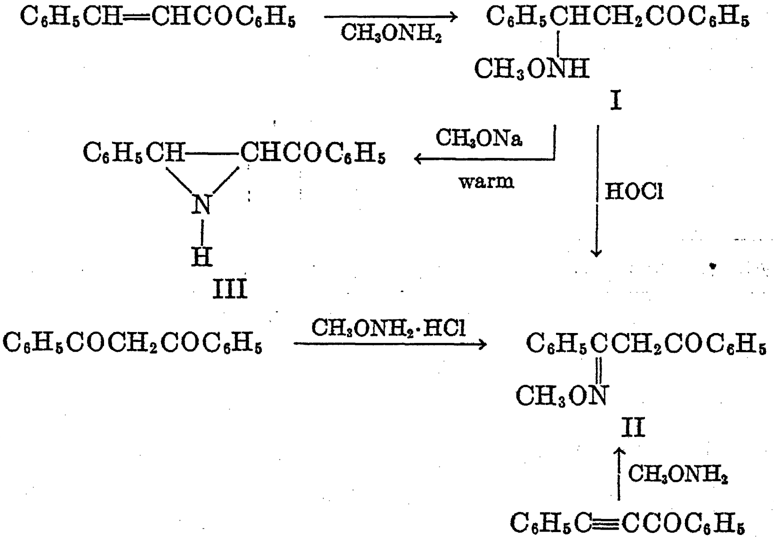
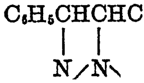


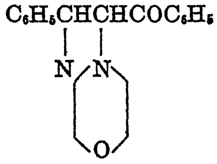
TABLE 12

Mixed α,β-diamino ketones

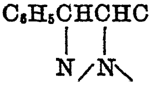


$C_6H_5CHCHCOC_6H_5$ (α-N, β-N)

β-AMINE	MELTING POINT	YIELD	REFERENCES
	°C.	*per cent*	
Morpholine	175 157	50	(31)
Tetrahydroquinoline	167	80	(32)
Tetrahydroisoquinoline	165	37	(38)
Cyclohexylamine	155	20	(38)
Thalline	160	85	(36)
N-Methylbenzylamine	140	36	(36)
Dibenzylamine	175	13	(36)
N-Methylethanolamine	108	10	(36)
8-Amino-6-methoxyquinoline	183	87	(40)

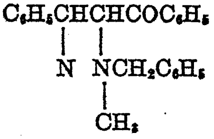


β-AMINE	MELTING POINT	YIELD	REFERENCES
Piperidine	Impure product		(31)
Tetrahydroquinoline	154	50	(32)
Tetrahydroisoquinoline	163	15	(38)
Thalline	143	68	(36)
8-Amino-6-methoxyquinoline	194	77	(40)
o-Methoxy-*N*-methylbenzylamine	174	10	(41)



$C_6H_5CHCHCOC_6H_5$ (α-N, β-N)

β-AMINE	MELTING POINT	YIELD	REFERENCES
Tetrahydroquinoline	149	35	(32)



$C_6H_5CHCHCOC_6H_5$ (β-N, α-$N(CH_3)CH_2C_6H_5$)

β-AMINE	MELTING POINT	YIELD	REFERENCES
Tetrahydroquinoline	153	50	(45)
8-Amino-6-methoxyquinoline	192	44	(40)

TABLE 12—*Continued*

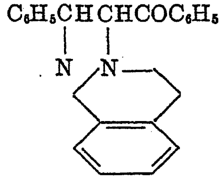
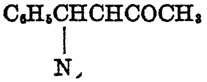
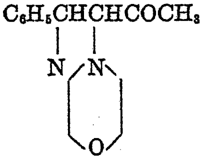
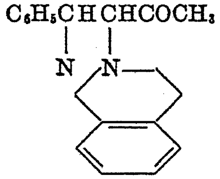
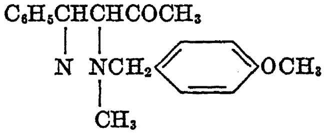
β AMINE	MELTING POINT	YIELD	REFERENCES
			
	°C.	*per cent*	
Morpholine	177	30	(38)
Tetrahydroquinoline	164	47	(38)
	 $\underset{\textstyle N,}{C_6H_5CH}CHCOCH_3$		
Morpholine	101 117	32	(38)
Tetrahydroquinoline	127	48	(37, 45)
Tetrahydroisoquinoline	148	40	(37)
6-Methoxytetrahydroquinoline	124	39	(36)
N-Methylbenzylamine	111	14	(36)
Dibenzylamine	160	4	(36)
N-Methylethanolamine	132	5	(36)
8-Amino-6-methoxyquinoline	160	66	(40)
			
Piperidine	123	10	(38)
Tetrahydroquinoline	173	51	(38)
Tetrahydroisoquinoline	135	<5	(37)
6-Methoxytetrahydroquinoline	126	40	(36)
8-Amino-6-methoxyquinoline	159	51	(40)
			
Piperidine	151	5	(37)
Morpholine	Impure product		(37)
Tetrahydroquinoline	109	44	(37)

TABLE 12—*Concluded*

β-AMINE	MELTING POINT	YIELD	REFERENCES
			
8-Amino-6-methoxyquinoline	173	90	(41)

The most interesting reaction of the addition products of type I is their loss of methyl alcohol on heating with sodium methoxide. Blatt wrote the resulting products as derivatives of α-aminobenzalacetophenone, $C_6H_5CH{=}C(NH_2)$-COC_6H_5. These products were identical with the products resulting from the reaction of ammonia with α,β-dibromobenzylacetophenones. For the reasons that have been presented in Section III,B,3, it now seems that these products have the ethylenimine type of structure (III).

The reactions of α,β-unsaturated ketones and their derivatives with amines of the carbonyl reagent type would seem to warrant a separate comprehensive review.

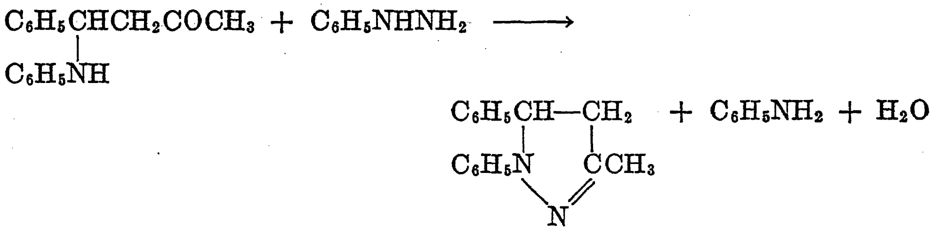
V. PROPERTIES OF AMINO KETONES

The amino ketones often serve as starting materials, or occur as intermediates, in the preparation of many interesting and valuable amino alcohols, diamines, heterocyclic nitrogen compounds, etc. Many of the reactions of α,β-unsaturated ketones and derivatives with amino compounds to form nitrogen ring compounds have been reviewed by Hollins in his excellent book (66). It is not the purpose of this review to survey completely all of such studies that have been reported, but merely to point out the various types of reactions, and to compare and contrast the properties of the various types of saturated and unsaturated amino ketones that have been mentioned previously in this review. Some of these reactions and properties already have been discussed in connection with structure proofs of the products.

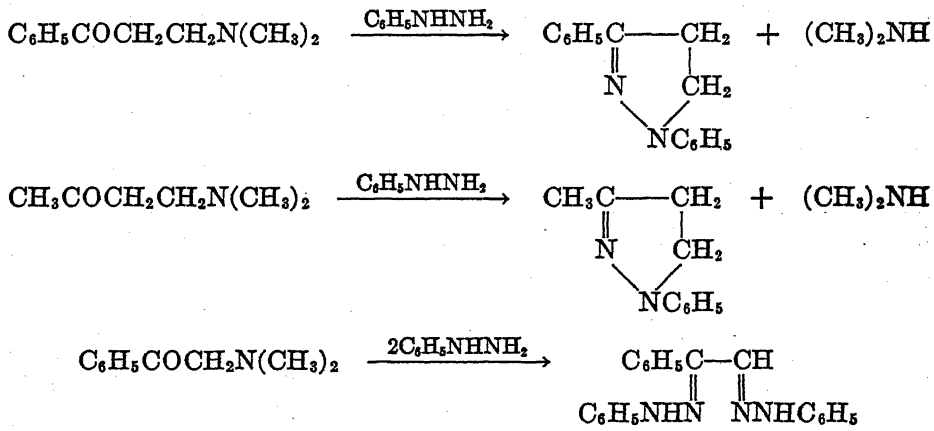
A. SATURATED AMINO KETONES

1. Monoamino ketones

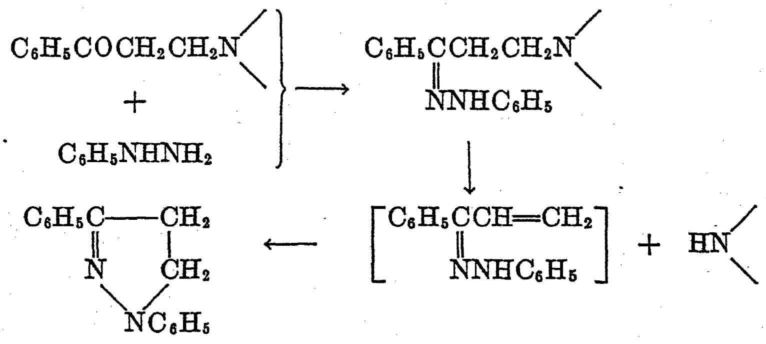
Because these molecules contain two reactive functional groups which are also activating groups, reactions dependent on active hydrogen as well as the usual reactions of the keto and amino groups are to be expected with such substances. In general, the α-aminoketones are more stable than the β-aminoketones at elevated temperatures or in the presence of acids, etc. The β-aminoketones, like β-amino acids, show a tendency to split out the amine and re-form the α,β-unsaturated carbonyl compound (34, 44). In attempting to prepare the phenylhydrazone of β-anilinobenzylacetone, Macovski and Silberg (90) obtained only 3-methyl-1,5-diphenylpyrazoline.



Similar results have been reported by Jacob and Madinaveitia (68) with ω-dimethylaminopropiophenone, which reacted with phenylhydrazine in warm acetic acid to form 1,3-diphenylpyrazoline. β-Dimethylaminoethyl methyl ketone under these conditions gave 1-phenyl-3-methylpyrazoline. These workers also found the dimethylamino group to be labile even in the α-position. ω-Dimethylaminoacetophenone under the above conditions gave phenylglyoxal osazone.



Recently, Nisbet (95) has succeeded in preparing the intermediate phenylhydrazones of such compounds, using the mild conditions outlined by v. Auwers and Voss (8). Furthermore, Nisbet showed that such phenylhydrazones could be converted, by heating with sodium acetate in a 50 per cent acetic acid solution, into the 1,3-diphenylpyrazoline obtained by Jacob and Madinaveitia (68). The postulated intermediate phenylhydrazones of the α,β-unsaturated ketones were not isolated, however.



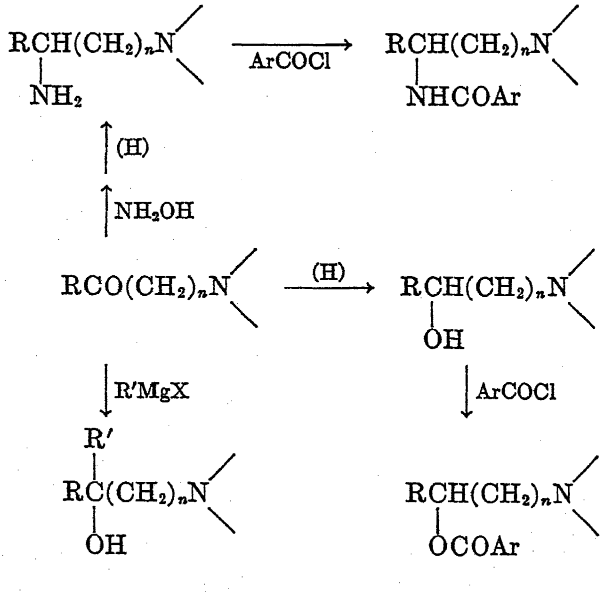
Several investigators have reduced certain α-amino and β-amino ketones to the corresponding amino alcohols, using sodium amalgam (44, 62, 77, 79), aluminum isopropoxide (21), or various catalytic methods (2, 67, 102) in attempts to produce pharmacologically active substances. Hartung (58, 59) has discussed the pressor activity of certain α-amino ketones and alcohols in two review articles.

Because of the unstable nature of some β-amino ketones toward catalytic reduction, better yields may often be obtained by reducing the β-amino ketone hydrochloride with sodium amalgam in water solution at −3°C. (44, 79).

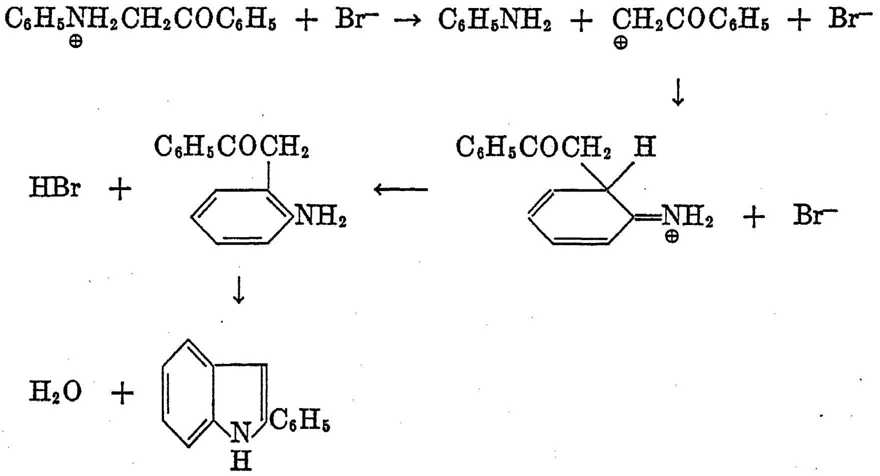
Amino alcohols of possible pharmacological interest may be prepared by adding Grignard reagents to either α-amino (76, 112, 115) or β-amino ketones (34, 80) to obtain the corresponding amino tertiary alcohols.

The keto group in the amino ketones often reacts readily with hydroxylamine to give oximes which may be reduced to the corresponding diamines (34, 39, 44, 57, 82). It has been pointed out by Cromwell and Hoeksema (39) that better yields are obtained from these reductions by the use of sodium and alcohol than by catalytic hydrogenation. These workers reported that such diamines are unstable at elevated temperatures and undergo self-condensation through loss of ammonia during distillation.

The amino alcohols and the diamines prepared from the amino ketones have been converted by aroyl chlorides into amino benzoates (44, 58) and amino benzamides (34, 39, 44), several of which have shown activity as local anesthetics.



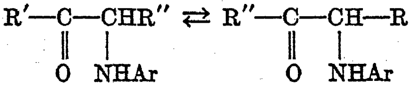
It has long been known that phenacyl halides react with aromatic primary amines to give not only α-amino ketones, but indoles as well. The indoles apparently result from the further reaction of the primary product, the α-amino ketone. The formation of indoles from such reactions has been reinvestigated and the earlier work reviewed recently by Crowther and Mann (46). These investigators have offered some evidence for the following mechanism[1], but they did not isolate the important postulated intermediate, o-aminobenzyl phenyl ketone.


$C_6H_5\overset{\oplus}{N}H_2CH_2COC_6H_5 + Br^- \rightarrow C_6H_5NH_2 + \overset{\oplus}{C}H_2COC_6H_5 + Br^-$
$C_6H_5COCH_2$
H
$=\overset{\oplus}{N}H_2$
$+ Br^-$
$HBr +$
NH_2
$H_2O +$
N
H
C_6H_5

Stevens and his coworkers (110, 111, 112, 113) have carried out an interesting series of investigations, starting with phenacyl bromide and ending with an α-amino-α,β-unsaturated ketone. Phenacyl bromide reacted with N-dimethylbenzylamine to give a quaternary ammonium bromide which was rearranged with sodium hydroxide to form α-dimethylaminobenzylacetophenone. This latter compound underwent a remarkable oxidation. In an alcohol solution containing sodium ethoxide, α-dimethylaminobenzylacetophenone was oxidized by air at 70°C. to α-dimethylaminobenzalacetophenone. The structures of all of these products were proved and mechanisms offered for these reactions.

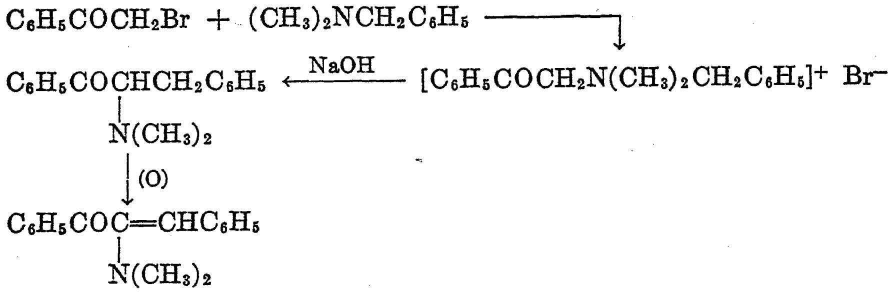
---

[1] Just after this review was submitted to the Editor there appeared an interesting paper by P. L. Julian *et al.* (J. Am. Chem. Soc. 67, 1203 (1945)), disputing the mechanism proposed by Crowther and Mann and surveying the various proposals of others. The complete explanation of these complex reactions is not yet at hand and must await further definitive experimentation. Julian points out that the reaction of an α-bromo ketone and an arylamine is a complicated system in the presence of oxygen and may involve any or all of the following: (*a*) direct replacement of the bromine; (*b*) addition to the carbonyl double bond; (*c*) rearrangement of the anilino ketones



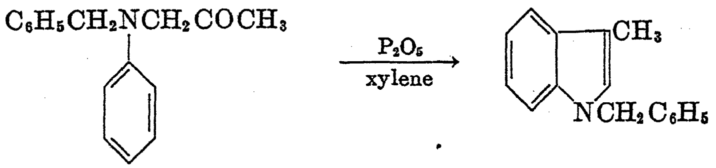
$$R'-\underset{\underset{O}{\|}}{C}-\underset{\underset{NHAr}{|}}{C}HR'' \rightleftarrows R''-\underset{\underset{O}{\|}}{C}-\underset{\underset{NHAr}{|}}{C}H-R'$$

(*d*) monoanil formation; (*e*) dianil formation; (*f*) anilineanil or diamine formation; (*g*) cleavage of arylamine from the diamine to form the indole.

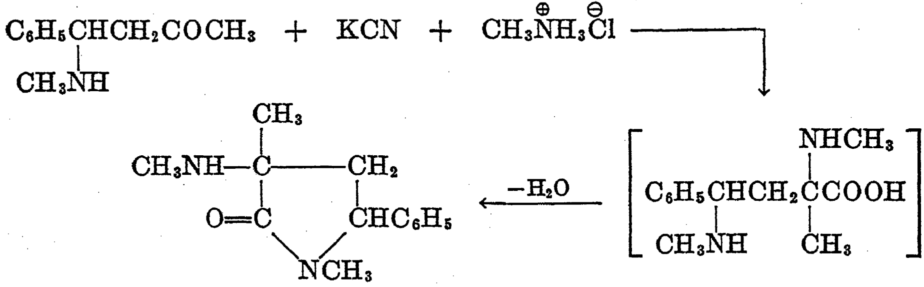


$$C_6H_5COCH_2Br + (CH_3)_2NCH_2C_6H_5 \rightarrow [C_6H_5COCH_2N(CH_3)_2CH_2C_6H_5]^+ Br^- \xrightarrow{NaOH} C_6H_5COCH(N(CH_3)_2)CH_2C_6H_5 \xrightarrow{(O)} C_6H_5COC(N(CH_3)_2){=}CHC_6H_5$$

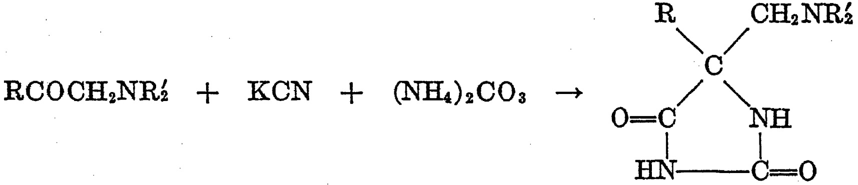
Bauer and Buhler (10) have reported an interesting cyclization of α-*N*-benzylanilinoacetone to form an indole.



Kohn (81) reported the formation of amino lactams from a Strecker type of reaction with β-amino ketones.



Another modification of the Strecker process, known as the Bucherer method for preparing hydantoins, has been used by Henze with α-amino ketones (63, 64, 91).



2. Diamino ketones

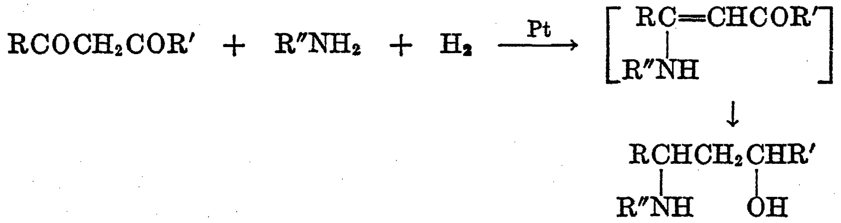
The diamino ketones do not appear to be as reactive as either the α- or β-amino ketones. The presence of the comparatively large hindering amino groups on both the α- and β-positions in the same molecule seems to hinder some of the usual carbonyl reactions of such substances (40, 41). As was pointed out in Section III,B,1, certain of these compounds will add Grignard reagents to form α,β-diamino tertiary alcohols. The hydrolysis of the α,β-diamino ketones in acid solution to form α-amino ketones was mentioned in Sections III,B,1 and III,B,2.

Some of these diamino ketones have been found to possess mild avian antimalarial activity (40).

B. UNSATURATED AMINO KETONES

Both the α-amino- and the β-amino-α,β-unsaturated ketones are readily hydrolyzed by acid solutions to diketones. The former give 1,2-diketones, while the latter yield 1,3-diketones as discussed in Section III,B,1. The reduction of an α-amino-α,β-unsaturated ketone to a saturated α-amino ketone and the reactions of both of these ketones with a Grignard reagent were also discussed in Section III,B,1.

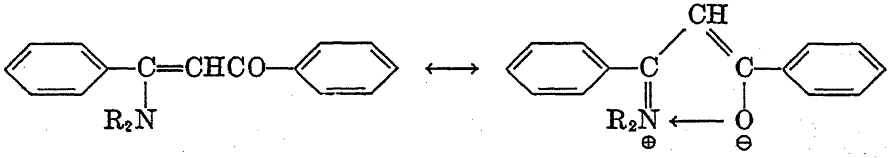
Skita and Keil (107) have reported the preparation of β-amino alcohols by a method that seems to involve the intermediate formation of β-amino-α,β-unsaturated ketones. A mixture of a 1,3-diketone and an amine was reduced with hydrogen in the presence of platinum.

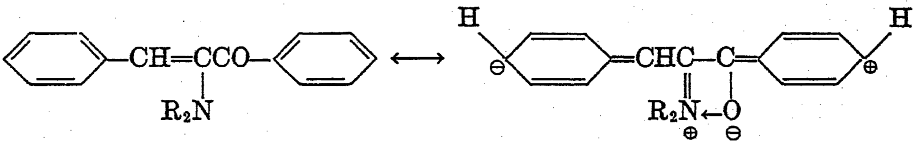


$$RCOCH_2COR' + R''NH_2 + H_2 \xrightarrow{Pt} \left[\begin{array}{l} RC{=}CHCOR' \\ \;| \\ R''NH \end{array}\right] \longrightarrow \begin{array}{l} RCHCH_2CHR' \\ \;|\qquad\;\;| \\ R''NH\;\;\; OH \end{array}$$

It has been pointed out at the beginning of Section III,A,3 that β-amino-α,β-unsaturated ketones undergo alkylation and acylation. More extensive studies, such as those that have been carried out with β-amino-α,β-unsaturated esters (84, 85), would be of interest and are contemplated.

A comparison of the color and constitution of α-amino- and β-amino-α,β-unsaturated ketones has been made by Cromwell and Johnson (42, 43). The β-amino-α,β-unsaturated ketones are colorless and show maximum absorption bands at about 3500 Å. with extinction coefficients of about 15–20 × 10^3. For the α-amino-α,β-unsaturated ketones, which range in color from yellow to deep red, the maximum absorption band was found at about 4000 Å. and with extinction coefficients of about 2–3 × 10^3 (see table 10). This difference in the absorption spectra was attributed to a difference in the nature of the resonance oscillators of such substances, approximated as follows:





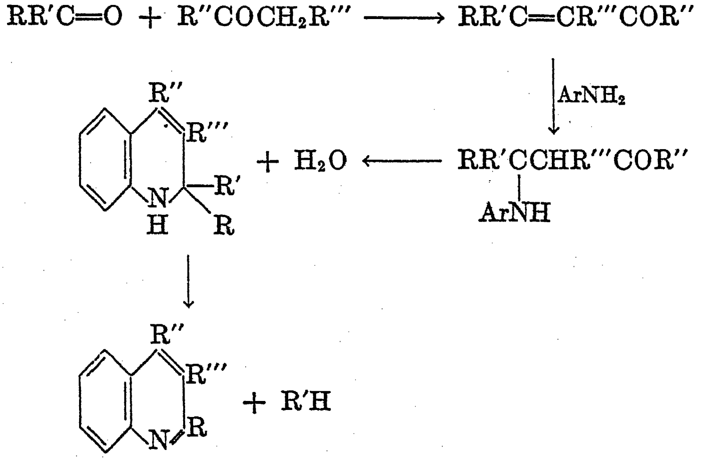
C. QUINOLINE SYNTHESES

One of the most important properties of the β-arylamino carbonyl compounds is their ability to cyclize to form substituted quinolines. Many reviews dealing with the various methods of preparing quinolines are available, and several of them include methods involving the cyclization of saturated and unsaturated β-arylamino ketones (12, 66, 69, 70, 92, 100).

1. From vinyl ketones

In 1885 Reed (98) heated a mixture of β-naphthylamine hydrochloride, acetone, and paraldehyde to obtain a dimethylnaphthoquinoline melting at 126–127°C. During the next few years Beyer (13) extended this method, using many various aldehydes, ketones, and arylamines to obtain several substituted quinolines. At about the same time, Engler and Riehm (54) reported the synthesis of 2,4-dimethylquinoline by heating a mixture of aniline hydrochloride with acetone for several days. The reaction may also be carried out by heating mesityl oxide with aniline hydrochloride. It is remarkable that in these reactions water and a hydrocarbon (in this case, methane) are eliminated during cyclization.

Beyer (13) did not isolate the intermediates in these reactions but postulated the intermediate formation of α,β-unsaturated ketones which then added the arylamine to form a β-arylamino ketone, which finally cyclized with loss of water and hydrogen or a hydrocarbon.



Since these early studies, the so-called Beyer method and its various modifications have been applied to the synthesis of many substituted quinolines (see page 262 of reference 66). The yields are usually not high, because of the requirement for high temperatures to cause hydrogen or the hydrocarbon to split out. John and his coworkers (69, 70) obtained very low yields of the expected 2-phenyl-4-methylquinolines on heating benzalacetone with various substituted aniline hydrochlorides at 135°C. for 5 hr. (see table 13). These workers reported that addition of an oxidant to facilitate removal of the hydrogen did not increase the yields.

Recently Campbell and Schaffner (22) have reported that the addition of an oxidant and the employment of milder conditions does give better yields in such

TABLE 13

2-Phenyl-4-methylquinolines from benzalacetone (69, 70)

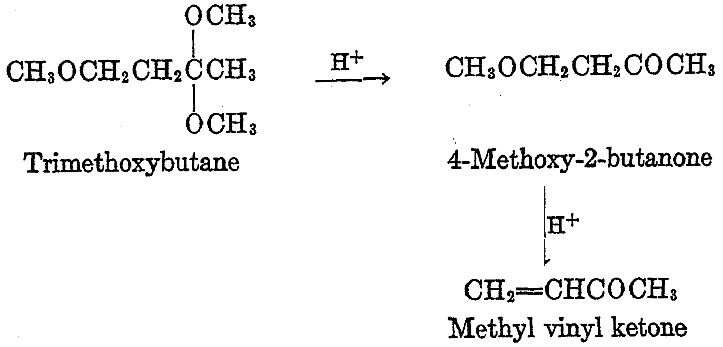
ARYLAMINE	2-PHENYL-4-METHYLQUINOLINES	MELTING POINT	YIELD
		°C.	*per cent*
Anisidine	6-Methoxy	129	8.8
Phenetidine	6-Ethoxy	153	5.3
p-Aminophenol	6-Hydroxy	212	7.7
p-Toluidine	6-Methyl	93	8.2
o-Toluidine	8-Methyl	91.5	14.3
o-Aminophenol	8-Hydroxy	58	10.6
o-Anisidine	8-Methoxy	98	13
o-Phenetidine	8-Ethoxy	98	14.4
p-Chloroaniline	6-Chloro	91.5	2.8
p-Bromoaniline	6-Bromo	100	3.8
p-Iodoaniline	6-Iodo	50	<2
p-Aminoethylbenzene	6-Ethyl	74	15
p-Xylidine	5,8-Dimethyl	111.5	7.1
ψ-Cumidine	5,6,8-Trimethyl	128	6.2
p-Dimethylaminoaniline	6-Dimethylamino	132	1.4
o-Aminobenzoic acid	8-Carboxy	202	8.7
p-Aminobenzoic acid	6-Carboxy	225	5

TABLE 14

Lepidines from vinyl ketones and derivatives (*22*)

ARYLAMINE	KETONE OR DERIVATIVE	LEPIDINES	BOILING POINT OR MELTING POINT	YIELD
			°C.	*per cent*
Aniline	Methylvinyl ketone	Lepidine	266/760 mm.	73
p-Anisidine	Methylvinyl ketone or Trimethoxybutane	6-Methoxy	122/2 mm.	52
p-Toluidine	Methylvinyl ketone	6-Methyl	104/2 mm.	65
o-Chloroaniline	Trimethoxybutane	8-Chloro	107	23
β-Naphthylamine	Trimethoxybutane	5,6-Benzo	101	58
Aniline	3-Penten-2-one	2-Methyl	105/4 mm.	62

syntheses. These workers refluxed for 2 hr. alcohol solutions of 0.625 mole of the arylamine hydrochloride with 1 mole of ferric chloride hexahydrate, 10 g. of zinc chloride, and 0.5 mole of either methyl vinyl ketone, or 4-methoxy-2-butanone, or trimethoxybutane to obtain very good yields of lepidines (see table 14). The relationship between these latter reagents is readily seen.



$$CH_3OCH_2CH_2\underset{OCH_3}{\overset{OCH_3}{C}}CH_3 \xrightarrow{H^+} CH_3OCH_2CH_2COCH_3$$

Trimethoxybutane

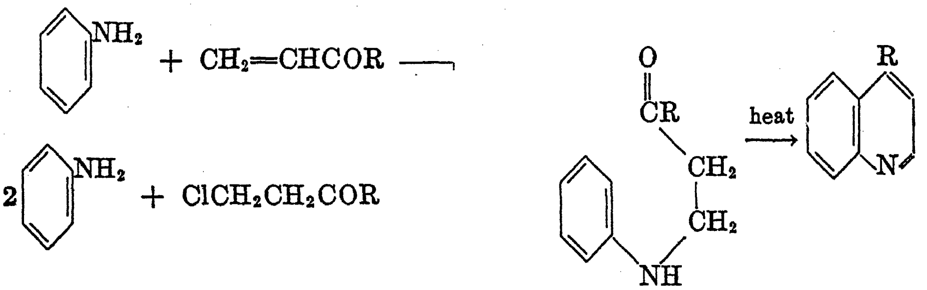
4-Methoxy-2-butanone

$$\downarrow H^+$$

$$CH_2{=}CHCOCH_3$$

Methyl vinyl ketone

Blaise and Maire (17) isolated the β-amino ketones from the reactions of aniline, aniline hydrochloride mixtures with either vinyl alkyl ketones or the β-chloro derivatives, and then cyclized these products by heating them in the presence of aniline hydrochloride, to form the substituted quinolines:



2. *From β-diketones*

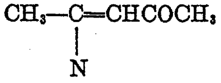
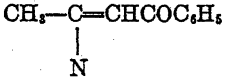
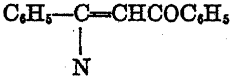
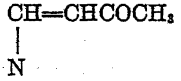
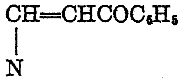
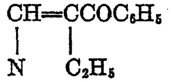
Earlier in this review (Section III,A,1) it was pointed out that almost the first reactions studied of amines with 1,3-diketones involved the use of arylamines. As has been indicated in Section III,A,1, Beyer (14) was actually the first to report the synthesis of quinolines by the cyclization of the resulting β-arylamino-α,β-unsaturated ketones. This excellent method for preparing 2,4-disubstituted quinolines has come to be known as the Combes method, but Combes (25) did not report the cyclization of his anilide of acetylacetone to give 2,4-dimethylquinoline until after the previous publication by Beyer (14) had appeared.

Since cyclization involves only the loss of water, and removal of hydrogen as in the Beyer method is not required, the yields reported from quinoline syntheses by the Combes method are often quite high. This method for preparing quinolines has been discussed by Hollins (66), and on page 269 of his book are listed the various 2,4-disubstituted quinolines prepared in this way through 1923.

In 1927, Roberts and Turner (100) published an excellent review of previous experiences with the Combes method and reported on an extensive series of investigations of the factors which affect the yields of substituted quinolines from these reactions. These investigations brought out the fact that, while most simple substituted anilines condense readily with acetylacetone to give good yields of the intermediate β-arylamino-α,β-unsaturated ketones, the second stage of the Combes quinoline synthesis (ring closure) is more definitely affected by the nature of the substituents present on the aniline benzene ring.

TABLE 15

Quinolines from β-arylamino-α,β-unsaturated ketones

AMINE	MELTING POINT OR BOILING POINT	QUINOLINES	MELTING POINT OR BOILING POINT	YIELD	REFERENCES
	 $\mathrm{CH_3{-}\underset{N}{\underset{\vert}{C}}{=}CHCOCH_3}$				
	°C.		°C.	per cent	
m-Anisidine	178/10 mm.	2,4-Dimethyl-7-methoxy	185/25 mm.	50	(86)
2,3,4-Trimethoxyaniline	70	2,4-Dimethyl-6,7,8-trimethoxy	59.5	>90	(87)
3,4,5-Trimethoxyaniline	101	2,4-Dimethyl-5,6,7-trimethoxy	59	>90	(87)
8-Aminoquinoline	95	Would not cyclize			(60)
	 $\mathrm{CH_3{-}\underset{N}{\underset{\vert}{C}}{=}CHCOC_6H_5}$				
3,4-Dimethoxyaniline	100	2-Methyl-4-phenyl-6,7-dimethoxy	142	80–90	(88)
	 $\mathrm{C_6H_5{-}\underset{N}{\underset{\vert}{C}}{=}CHCOC_6H_5}$				
m-Anisidine	76	2,4-Diphenyl-7-methoxy	102	77	(86)
	 $\mathrm{\underset{N}{\underset{\vert}{CH}}{=}CHCOCH_3}$				
Aniline	Not isolated	2-Methyl	246/760 mm.		(101)
p-Toluidine	Not isolated	2,6-Dimethyl	55		(101)
	 $\mathrm{\underset{N}{\underset{\vert}{CH}}{=}CHCOC_6H_5}$				
Aniline	Not isolated	2-Phenyl	62	25	(93)
	 $\mathrm{\underset{N}{\underset{\vert}{CH}}{=}\underset{C_2H_5}{\underset{\vert}{C}}COC_6H_5}$				
Aniline	Not isolated	2-Phenyl-3-ethyl	63	40	(93)

In agreement with the predictions of Roberts and Turner (100), several other investigators have since reported the preparation of various substituted quinolines using anilines with alkoxy substituents on the benzene ring in certain favorable arrangements. When only one alkoxy group is present in the ortho- or para-position of the aniline, the β-arylamino-α-β-unsaturated ketones (which can be obtained in good yields) cannot be induced to cyclize to the expected quinolines (100). The presence of at least one methoxyl group in the meta-position, however, seems to greatly facilitate ring closure (see table 15).

A modification (93, 101) of the Combes method involves the use of β-hydroxy-α,β-unsaturated ketones of the type RCOCR=CHOH (see table 15).

Borsche (19) has prepared certain tricyclic quinolines, using arylamines and β-diketones in which one ketone group is in an alicyclic ring (see reference 66, pages 372–4 and 387 for discussion).

Some of the material covered by this review has become available to the author during the past six years at the University of Nebraska, through the efforts of his several graduate research students whose names appear as coauthors of the papers mentioned here. The author is particularly indebted to his wife, Mary K. Cromwell, who helped in the literature search and typed the manuscript.

VI. REFERENCES

(1) ALGAR, J., HICKEY, A., AND SHERRY, P. G.: Proc. Roy. Irish Acad. **49B**, 109 (1943).
(2) ALLEWELT, A. L., AND DAY, A. R.: J. Org. Chem. **6**, 384 (1941).
(3) ANDRÉ, E.: Compt. rend. **152**, 525 (1911).
(4) v. AUWERS, K., AND MULLER, H.: J. prakt. Chem. **137**, 57 (1933).
(5) v. AUWERS, K., AND MULLER, H.: J. prakt. Chem. **137**, 81 (1933).
(6) v. AUWERS, K., AND MULLER, H.: J. prakt. Chem. **137**, 102 (1933).
(7) v. AUWERS, K., AND SUSEMIHL, W.: Ber. **63**, 1072 (1930).
(8) v. AUWERS, K., AND VOSS, H.: Ber. **42**, 4411 (1909).
(9) BASU, U.: J. Indian Chem. Soc. **8**, 119 (1931); Chem. Abstracts **25**, 4881 (1931).
(10) BAUER, K. H., AND BUHLER, K.: Arch. Pharm. **262**, 128 (1924); Chem. Abstracts **18**, 3188 (1924).
(11) BENARY, E.: Ber. **63B**, 1573 (1930).
(12) BERGSTROM, F. W.: Chem. Rev. **35**, 150 (1944).
(13) BEYER, C.: J. prakt. Chem. **33**, 393 (1886).
(14) BEYER, C.: Ber. **20**, 1767 (1887).
(15) BEYER, C.: Ber. **24**, 1662 (1891).
(16) BEYER, C., AND CLAISEN, L.: Ber. **20**, 2178 (1887).
(17) BLAISE, E. E., AND MAIRE, M.: Bull. soc. chim. [4] **3**, 658 (1908).
(18) BLATT, A. H.: J. Am. Chem. Soc. **61**, 3494 (1939).
(19) BORSCHE, W.: Ann. **377**, 70 (1910).
(20) BRANCH, G., AND CALVIN, M.: *The Theory of Organic Chemistry*, Prentice-Hall, Inc., New York (1941).
(21) BURGER, A., AND HARNEST, G. H.: J. Am. Chem. Soc. **65**, 2382 (1943).
(22) CAMPBELL, K. N., AND SCHAFFNER, I. J.: J. Am. Chem. Soc. **67**, 86 (1945).
(23) COLLIE, J. N.: Ann. **226**, 294 (1884).
(24) COMBES, A.: Ann. chim. phys. [6] **12**, 245 (1887).
(25) COMBES, A.: Compt. rend. **106**, 142 (1888); Bull. soc. chim. [2] **49**, 89 (1888).

(26) Combes, A., and Combes, C.: Bull. soc. chim. [3] **7**, 778 (1892).
(27) Conant, J. B., and Thompson, A. F., Jr.: J. Am. Chem. Soc. **54**, 4039 (1932).
(28) Cromwell, N. H.: J. Am. Chem. Soc. **62**, 1672 (1940).
(29) Cromwell, N. H.: J. Am. Chem. Soc. **62**, 2897 (1940).
(30) Cromwell, N. H.: J. Am. Chem. Soc. **62**, 3470 (1940).
(31) Cromwell, N. H.: J. Am. Chem. Soc. **63**, 837 (1941).
(32) Cromwell, N. H.: J. Am. Chem. Soc. **63**, 2984 (1941).
(33) Cromwell, N. H., Babson, R. D., and Harris, C. E.: J. Am. Chem. Soc. **65**, 312 (1943).
(34) Cromwell, N. H., and Burch, J. S.: J. Am. Chem. Soc. **66**, 872 (1944).
(35) Cromwell, N. H., and Caughlan, J. A.: J. Am. Chem. Soc. **67**, 2235 (1945).
(36) Cromwell, N. H., Caughlan, J. A., and Gilbert, G. R.: J. Am. Chem. Soc. **66**, 401 (1944).
(37) Cromwell, N. H., and Cram, D. J.: J. Am. Chem. Soc. **65**, 301 (1943).
(38) Cromwell, N. H., Harris, C. E., and Cram, D. J.: J. Am. Chem. Soc. **66**, 134 (1944).
(39) Cromwell, N. H., and Hoeksema, H.: J. Am. Chem. Soc. **66**, 870 (1944).
(40) Cromwell, N. H., and Hoeksema, H.: J. Am. Chem. Soc. **67**, 124 (1945).
(41) Cromwell, N. H., and Hoeksema, H.: J. Am. Chem. Soc. **67**, 1658 (1945).
(42) Cromwell, N. H., and Johnson, R. S.: J. Am. Chem. Soc. **65**, 316 (1943).
(43) Cromwell, N. H., and Johnson, R. S.: J. Am. Chem. Soc. **65**, 2481 (1943).
(44) Cromwell, N. H., Wiles, Q. T., and Schroeder, O. C.: J. Am. Chem. Soc. **64**, 2432 (1942).
(45) Cromwell, N. H., and Witt, I. H.: J. Am. Chem. Soc. **65**, 308 (1943).
(46) Crowther, A. F., and Mann, F. G.: J. Chem. Soc. **1943**, 58.
(47) Darapsky, A., and Spannagel, H.: J. prakt. Chem. [2] **92**, 272 (1915).
(48) Davis, H. L.: J. Am. Chem. Soc. **63**, 1677 (1941).
(49) Décombe, J.: Ann. chim. **18**, 81 (1932).
(50) Dufraisse, C., and Moureu, H.: Bull. soc. chim. [4] **41**, 457, 850 (1927).
(51) Dufraisse, C., and Netter, R.: Bull. soc. chim. [4] **51**, 550 (1932).
(52) Dupont, G.: Bull. soc. chim. [4] **41**, 1167 (1927).
(53) Dutch patent 47,778 (February 15, 1940); Chem. Abstracts **34**, 6301 (1940). U. S. patent 2,198,260 (April 23, 1940); Chem. Abstracts **34**, 5463 (1940).
(54) Engler, C., and Riehm, P.: Ber. **18**, 2245 (1885).
(55) Fisher, E., and Bülow, C.: Ber. **18**, 2131 (1885).
(56) Georgi, R., and Schwyzer, A.: J. prakt. Chem. **86**, 273 (1912).
(57) Harries, C.: Ber. **34**, 300 (1901).
(58) Hartung, W. H.: Chem. Rev. **9**, 389 (1931).
(59) Hartung, W. H.: Ind. Eng. Chem. **37**, 126 (1945).
(60) Hazlewood, S. J., Hughes, G. K., and Lions, F.: J. Proc. Roy. Soc. N.S. Wales **71**, 462 (1938); Chem. Abstracts **33**, 610 (1939).
(61) Heintz, W.: Chem. Zentr. **1874**, 372.
(62) Heintz, W.: Ann. **183**, 290 (1876).
(63) Henze, H. R., and Holder, C. B.: J. Am. Chem. Soc. **63**, 1943 (1941).
(64) Henze, H. R., and Humphreys, D. D.: J. Am. Chem. Soc. **64**, 2881 (1942).
(65) Hochstetter, A., and Kohn, M.: Sitzber. Akad. Wiss. Wien **112^{2}B**, 614 (1903); Monatsh. **24**, 773 (1903).
(66) Hollins, C.: *The Synthesis of Nitrogen Ring Compounds.* Ernest Benn, Ltd., London (1924).
(67) Immediata, T., and Day, A. R.: J. Org. Chem. **5**, 512 (1940).
(68) Jacob, A., and Madinaveitia, J.: J. Chem. Soc. **1937**, 1929.
(69) John, H., and Fr. Noziczka: J. prakt. Chem. [2] **111**, 65 (1925).
(70) John, H., and Weber, G.: J. prakt. Chem. [2] **111**, 83 (1925).
(71) Jones, E. C. S., and Kerner, J.: J. Chem. Soc. **1933**, 363.
(72) Knoevenagel, E., and Faber, W.: Ber. **31**, 2773 (1898).

(73) Knorr, L., and Rossler, K.: Ber. **36,** 1278 (1903).
(74) Kohler, E. P., and Addinall, C. R.: J. Am. Chem. Soc. **52,** 3728 (1930).
(75) Kohler, E. P., and Blatt, A. H.: J. Am. Chem. Soc. **50,** 1217 (1928).
(76) Kohler, E. P., and Bruce, W. F.: J. Am. Chem. Soc. **53,** 1994 (1931).
(77) Kohn, M.: Sitzber. Akad. Wiss. Wien **112^{2}B,** 944 (1903); Monatsh. **25,** 135 (1904).
(78) Kohn, M.: Sitzber. Akad. Wiss. Wien **113^{2}B,** 445 (1904); Monatsh. **25,** 817 (1904).
(79) Kohn, M.: Sitzber. Akad. Wiss. Wien **116^{2}B,** 321 (1907); Monatsh. **28,** 423 (1907).
(80) Kohn, M.: Sitzber. Akad. Wiss. Wien **116^{2}B,** 953 (1907); Monatsh. **28,** 1049 (1907).
(81) Kohn, M.: Sitzber. Akad. Wiss. Wien **117^{2}B,** 243 (1908); Monatsh. **29,** 497 (1908).
(82) Kohn, M., and Morgenstern, O.: Sitzber. Akad. Wiss. Wien **117^{2}B,** 265 (1908); Monatsh. **29,** 519 (1908).
(83) Kuckert, O.: Ber. **18,** 618 (1885).
(84) Lauer, W., and Cromwell, N. H.: J. Am. Chem. Soc. **64,** 612 (1942).
(85) Lauer, W., and Lones, G. W.: J. Am. Chem. Soc. **59,** 232 (1937).
(86) Lempert, H., and Robinson, R.: J. Chem. Soc. **1934,** 1419.
(87) Lions, F.: J. Proc. Roy. Soc. N.S. Wales **63,** 159 (1930); Chem. Abstracts **24,** 5300 (1930).
(88) Lions, F.: J. Proc. Roy. Soc. N.S. Wales **71,** 242 (1938); Chem. Abstracts **32,** 7460 (1938).
(89) Lutz, R. E.: University of Virginia, private communication.
(90) Macovski, E., and Silberg, A.: J. prakt. Chem. **137,** 131 (1933).
(91) Magee, J. W., and Henze, H. R.: J. Am. Chem. Soc. **60,** 2148 (1938).
(92) Manske, R. H.: Chem. Rev. **30,** 118 (1942).
(93) Montagne, M., and Roch, M.: Compt. rend. **213,** 620 (1941); Chem. Abstracts **38,** 6286 (1944).
(94) Moureu, H.: Ann. chim. [10] **14,** 283 (1930).
(95) Nisbet, H. B.: J. Chem. Soc. **1945,** 126.
(96) *Organic Syntheses*, Collective Volume I, 2nd edition, p. 196. John Wiley and Sons, Inc., New York (1941).
(97) Pirrone, F.: Gazz. chim. ital. **65,** 909 (1935); **66,** 429 (1936).
(98) Reed, J. H.: J. prakt. Chem. **32,** 630 (1885).
(99) Remick, A. E.: *Electronic Interpretations of Organic Chemistry*, p. 123. John Wiley and Sons, Inc., New York (1943).
(100) Roberts, E., and Turner, E. E.: J. Chem. Soc. **1927,** 1832.
(101) Romet, M.: Compt. rend. **200,** 1676 (1935).
(102) Rubin, N., and Day, A. R.: J. Org. Chem. **5,** 54 (1940).
(103) Rügheimer, L.: Ber. **47,** 2759 (1914).
(104) Rügheimer, L., and Ritter, G.: Ber. **45,** 1332 (1912).
(105) Ruhemann, S., and Watson, E. R.: J. Chem. Soc. **85,** 456 (1904).
(106) Ruhemann, S., and Watson, E. R.: J. Chem. Soc. **85,** 1170 (1904).
(107) Skita, A., and Keil, F.: Z. angew. Chem. **42,** 501 (1929).
(108) Smith, M. E., and Adkins, H.: J. Am. Chem. Soc. **60,** 407 (1938).
(109) Sokoloff, N., and Latschinoff, P.: Ber. **7,** 1384, 1776 (1874).
(110) Stevens, T. S.: J. Chem. Soc. **1930,** 2107.
(111) Stevens, T. S., Creighton, E. M., Gordon, A. B., and MacNicol, M.: J. Chem. Soc. **1928,** 3193.
(112) Stevens, T. S., and Hems, B. A.: J. Chem. Soc. **1937,** 856.
(113) Stevens, T. S., Snedden, W. W., Stiller, E. T., and Thomson, T.: J. Chem. Soc. **1930,** 2119.
(114) Stewart, V. E., and Pollard, C. B.: J. Am. Chem. Soc. **58,** 1980 (1936); **59,** 2702 (1937).
(115) Suter, C. M., and Weston, A. W.: J. Am. Chem. Soc. **64,** 2451 (1942).
(116) Tambor, J., and Wildi, F.: Ber. **31,** 349 (1898).

(117) Taylor, T. W. J., Owen, J. S., and Whittaker, D.: J. Chem. Soc. **1938**, 206.
(118) Watson, E. R.: J. Chem. Soc. **85**, 1319 (1904).
(119) Weissberger, A., and Bach, H.: Ber. **64**, 1095 (1931); **65**, 631 (1932).
(120) Wenker, H.: J. Am. Chem. Soc. **57**, 2328 (1935).
(121) Wheland, G. W.: *The Theory of Resonance*, pp. 208–10. John Wiley and Sons, Inc. New York (1944).
(122) Wieland, H.: Ber. **37**, 1150 (1904).
(123) Wittig, G.: Ber. **58**, 19 (1925).
(124) Wittig, G., and Blumenthal, H.: Ber. **60B**, 1085 (1927).

THE ULLMANN SYNTHESIS OF BIARYLS

PAUL E. FANTA[1]

Department of Chemistry, University of Rochester, Rochester, New York

Received August 10, 1945

CONTENTS

I. Introduction 139
II. Nature and scope of the reaction 140
  A. The aromatic halide 140
  B. Synthesis of unsymmetrical biaryls 141
  C. Intramolecular reactions 144
  D. Reactions of polyfunctional compounds 146
III. Experimental conditions 147
  A. Starting materials 147
  B. Proportions of reacting substances 148
  C. Temperature 148
  D. Diluents 149
  E. Protective atmosphere 149
  F. Agitation 149
  G. Isolation of products 149
IV. Mechanism 150
V. Tables 151
  Symmetrical biaryls (table 1) 152
  Unsymmetrical biaryls (table 2) 175
  Unsuccessful reactions (table 3) 186
VI. References 190

I. INTRODUCTION

A general method for the preparation of a biaryl consists in the condensation of two molecules of an aromatic halide in the presence of a metallic agent, with the elimination of metal halide:

$$RX + R'X + M \longrightarrow RR' + MX_2$$

The work of Fritz Ullmann (216, 219) and of subsequent investigators showed that copper is particularly effective in this type of condensation,[2] so that biaryl formation with the elimination of copper halide has come to be known as the Ullmann reaction.

The synthesis devised by Ullmann is of quite general applicability and has found wide use in the preparation of many symmetrical and unsymmetrical biaryls and polyaryls which would otherwise be difficult to obtain.

---

[1] Abbott Laboratories Fellow, 1943–44. The author is indebted to Dr. Robert B. Carlin for assistance in the preparation of the manuscript.

[2] The relative merits of various metals in this condensation are discussed in Houben's *Die Methoden der organischen Chemie*, Vol. II, pp. 786–800, Georg Thieme, Leipzig (1925), and in references 64 and 152.

II. NATURE AND SCOPE OF THE REACTION

A. *The aromatic halide*

The success of the Ullmann reaction is dependent upon the nature of the aromatic halide. Considering first the halogen residue, it has been observed that chlorine, bromine, iodine, and the halogenoid thiocyanate group (97) may be eliminated with biaryl formation. Closely related is the elimination of the disulfide linkage (97), according to the equation:

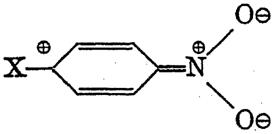
$$RSSR + Cu \longrightarrow RR + CuS$$

An aromatic fluorine atom has never been reported to be active in the Ullmann reaction.

The order of reactivity of the halogens is $I > Br > Cl$. In general, the latter two substituents undergo reaction only when activating groups are present in the aromatic nucleus. The amount of activation required in order that the Ullmann reaction may occur has been studied with a great variety of halogenated aromatic compounds by Mascarelli (132).

Substituents in the aromatic nucleus which affect the Ullmann reaction of halogenated benzene derivatives may be divided into four classes:

(*1*) *Activating groups:* Certain electronegative groups in the ortho- and para-positions with respect to the halogen atom activate the latter through the operation of a $-T$ effect[3] which leaves the carbon atom to which the halogen is attached with a residual positive charge. The nitro group is the most effective activator:



An ortho or para carbalkoxyl group or carbonyl group also activates markedly. Numerous examples of $-T$ activation by these groups may be found in table 1. Substituents such as an ortho halogen atom which might be expected to activate in a similar manner through the operation of a $-I$ effect actually have no detectable influence on reactivity. Thus 2,4,6-trichloroiodobenzene undergoes the Ullmann reaction no more readily than does iodobenzene (216).

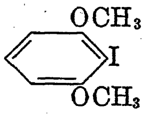
(*2*) *Deactivating groups:* Substituents which exert a $+T$ effect would be expected to lead to deactivation when they occupy positions ortho or para to the halogen atom. Evidence for this effect is not clear-cut (16, 216).

(*3*) *Inhibiting groups:* A decrease in yield of biaryl is generally observed when certain groups are present in the aromatic nucleus (216). Inhibition is particularly marked when groups such as $—NH_2$, $—NHCOCH_3$, $—NHCH_3$, $—SO_2NH_2$, $—SO_2NHC_6H_5$, $—COOH$, and $—OH$, which can give rise to amination, decarboxylation, or ether formation as a side reaction, are present. The nitrogen-containing groups must be protected by alkylation or acylation, the carboxyl group by esterification, and the hydroxyl group by etherification.

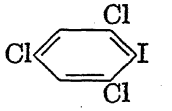
---

[3] The terminology used here is that of Remick, *Electronic Interpretations of Organic Chemistry*, John Wiley and Sons, Inc., New York (1943).

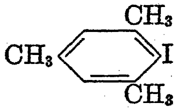
(4) *Steric effects:* In some cases bulky substituents in positions adjacent to the reactive halogen hinder biaryl formation. The relative hindrance of these groups in the Ullmann reaction is the same as their interference effect on the restriction of rotation in resolvable biphenyls.[4] For example, the yields of biaryl indicated were obtained from the following iodobenzene derivatives: 2,6-dimethoxyiodobenzene (II) (229), 2,4,6-trichloroiodobenzene (III) (216), and 2,4,6-trimethyliodobenzene (IV) (145, 216).



II



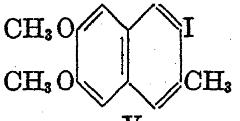
III



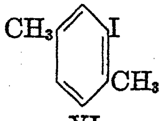
IV

(yield of biaryl 85–90%) (yield of biaryl 52.5%) (yield of biaryl very poor)

In other cases the effect is not predictable. Thus V could not be caused to undergo an Ullmann reaction under a variety of conditions (3), while a 90 per cent yield of the expected biaryl was obtained with VI (216).



V



VI

The generalizations regarding the effect of substituents on the Ullmann reaction of benzene derivatives may be applied to other aromatic compounds according to the usual concepts of aromaticity.

B. Synthesis of unsymmetrical biaryls[5]

The Ullmann reaction has been applied with considerable success to the synthesis of unsymmetrical biaryls. In addition to a consideration of the previously discussed conditions, the synthesis of an unsymmetrical biaryl requires the selection of an optimum ratio of the two component starting materials. By making certain simplifying assumptions, the conditions which are necessary for a successful crossed reaction can be deduced.

When a mixture of two aromatic halides, RX and R′X′, is subjected to the conditions of the Ullmann reaction, four competing reactions are possible:

$$RX + RX \longrightarrow R\text{—}R \qquad (1)$$

$$RX + R'X' \longrightarrow R\text{—}R' \qquad (2)$$

$$R'X' + RX \longrightarrow R'\text{—}R \qquad (3)$$

$$R'X' + R'X' \longrightarrow R'\text{—}R' \qquad (4)$$

---

[4] For references to the original literature see Shriner, Adams, and Marvel in *Organic Chemistry, an Advanced Treatise,* edited by Henry Gilman, Second Edition, Vol. I, p. 362, John Wiley and Sons, Inc., New York (1943).

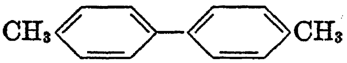
[5] For a discussion of other methods of synthesis of unsymmetrical biaryls see Bachmann and Hoffmann, *Organic Reactions,* edited by Roger Adams, Vol. II, pp. 241–3, John Wiley and Sons, Inc., New York (1944).

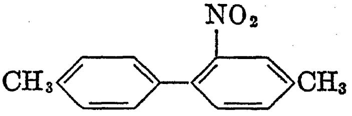
Depending upon the relative rates of these four reactions, a number of different courses for the over-all reaction may be deduced:

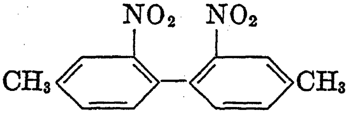
Case 1: The four reactions proceed at nearly equal rates. If this is true and equimolar quantities of the two component starting materials are used, the yield will be distributed among the various possible products approximately as follows:

$$\begin{array}{ccccccccc} \mathrm{RX} & + & \mathrm{R'X'} & \longrightarrow & \mathrm{RR} & + & \mathrm{RR'} & + & \mathrm{R'R'} \\ (1\text{ mole}) & & (1\text{ mole}) & & (\tfrac{1}{4}\text{ mole}) & & (\tfrac{1}{2}\text{ mole}) & & (\tfrac{1}{4}\text{ mole}) \end{array}$$

The success of the reaction depends upon the feasibility of separating the desired product from the symmetrical biaryls. This may be accomplished if the chemical or physical properties of the biaryls differ sufficiently. For example, Marler and Turner (123) subjected a mixture of 4-iodotoluene and 4-bromo-3-nitrotoluene to the conditions of the Ullmann reaction and obtained a mixture of the biphenyls VII, VIII, and IX.

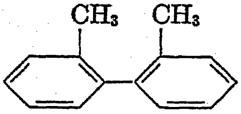

VII

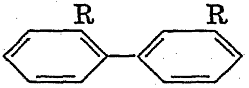

VIII


IX

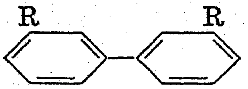
VII and VIII were separated from the dinitrobiphenyl IX by extraction with petroleum ether, after which VII and VIII were separated by fractional distillation.

A much more difficult case was encountered by Mayer and Freitag (136), who obtained the biphenyls X, XI, and XII by the Ullmann reaction of 2-iodo- and 3-iodotoluenes. Biphenyl X was separated from the mixture of products by repeated fractional distillation. The remaining mixture of XI and XII was oxidized to give a mixture of the corresponding diphenic acids XIII and XIV, which were separated by extraction with water.


X

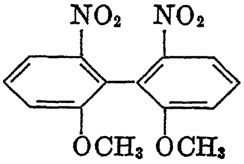

XI: R = CH_3

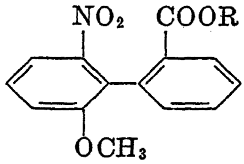
XIII: R = COOH

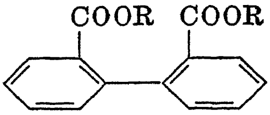

XII: R = CH_3

XIV: R = COOH

A difference in the number of polar groups provides a basis for a chemical separation. For example, Adams and Finger (2) obtained a mixture of the biphenyls XIV, XV, and XVI as the product of a crossed Ullmann reaction.

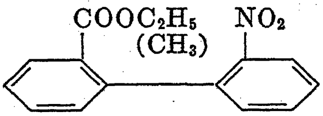

XIV

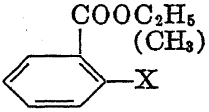

XV: R = CH_3
XVII: R = H

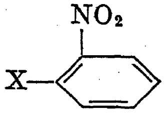

XVI: R = CH_3
XVIII: R = H

After saponification with sodium hydroxide solution, the neutral compound XIV was separated from a mixture of the sodium salts of the acids XVII and XVIII. Finally, the two acids were separated by fractional crystallization from benzene.

Case 2: Reaction 1 proceeds at a rapid rate compared to reactions 2, 3, and 4. In this case RX would be completely used up in the formation of RR. When the reaction is continued for a longer time or under more drastic conditions, R′X′ reacts to give R′R′. The symmetrical biaryls are the only products of the reaction. In such a case the formation of RR′ may be favored by changing either X or X′ in the starting materials, so that the aromatic halides are of more nearly equal reactivity. For example, the synthesis of XIX from XX and XXIII was accomplished in 20 per cent yield (156), while a 68 per cent yield was attained by the use of XXII and XXI (184a).

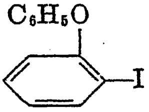

XIX

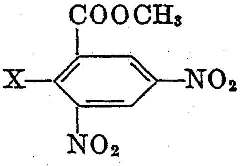

XX: X = Br
XXII: X = I

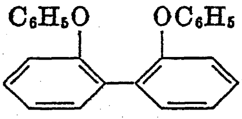

XXI: X = Br
XXIII: X = I

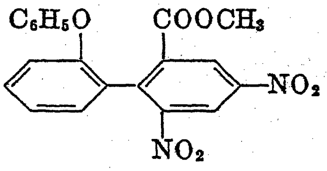
In the latter case the two components are of more nearly equal reactivity, since the lower activity of bromine as compared to iodine is compensated by the greater activation effect of the nitro group as compared to the carbalkoxyl group.

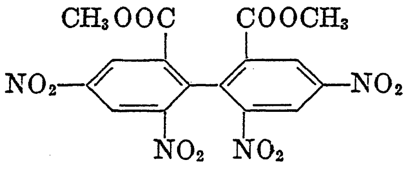
However, the same principle was unsuccessful when applied to the attempted preparation of biphenyl XXVIII (111).


XXIV


XXV: X = Cl
XXVI: X = Br


XXVII


XXVIII

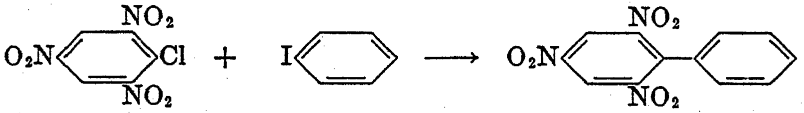


XXIX

In this case a reaction mixture containing XXIV and XXV gave only the symmetrical biphenyl XXVII. When the bromo derivative XXVI was used in the reaction instead of the chloro derivative, with the intention of making the reactivities of the two components more nearly equal, the reactivity of the dinitrocarbomethoxy nucleus was actually increased to such an extent that XXIX was the only product which could be isolated and again no unsymmetrical biphenyl was formed.

Another condition which favors unsymmetrical biaryl formation is the presence of an excess of the less reactive component. Numerous examples of the use of this technique are to be found in the literature (5, 67, 113, 155a).

Case 3: Reaction 2 or reaction 3 predominates. Most of the reactants are utilized in the formation of RR′, and relatively small amounts of the symmetrical biaryls are formed. In the most favorable cases the crossed reaction may be obtained with the complete exclusion of symmetrical products (110). An example of this case which has been carefully investigated (155a) is the reaction of picryl chloride and iodobenzene to give 2,4,6-trinitrobiphenyl as the only detectable product.



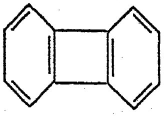
XXX Picryl chloride

XXXI Iodobenzene

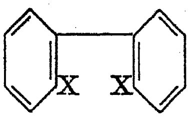
XXXII 2,4,6-Trinitrobiphenyl

C. Intramolecular reactions

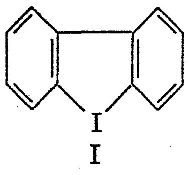
Several intramolecular Ullmann reactions have been described in the literature. Lothrop has reported (120, 121) the synthesis of biphenylene (XXXIII) and several of its derivatives by the treatment of appropriately substituted 2,2′-dihalogen biphenyls (XXXIV) or 2,2′-biphenyleneiodonium iodides (XXXV) with cuprous oxide.



XXXIII Biphenylene



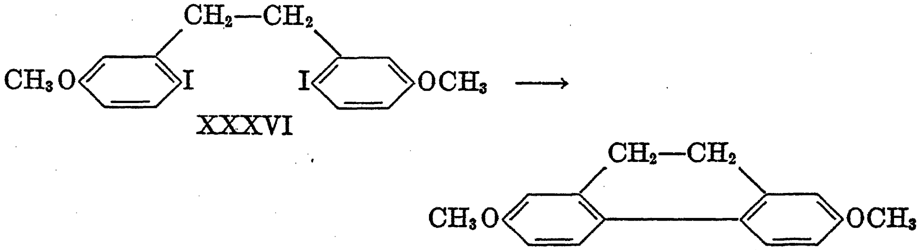
XXXIV



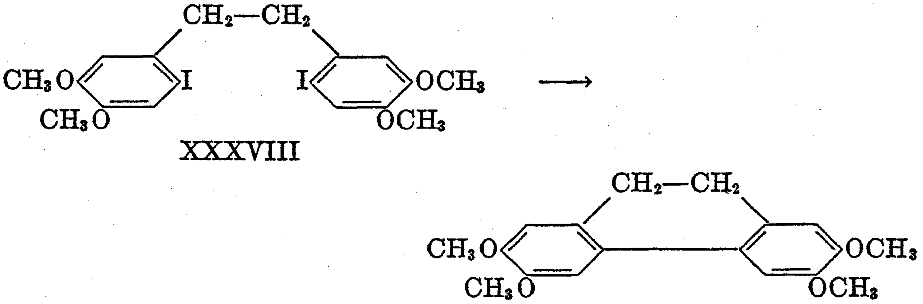
XXXV

The preparation of 2,7-dimethoxy-9,10-dihydrophenanthrene (XXXVII)

(43) and of 2,3,6,7-tetramethoxy-9,10-dihydrophenanthrene (XXXIX) (54) was accomplished by intramolecular Ullmann reactions involving the appropriately substituted dibenzyl derivatives XXXVI and XXXVIII.

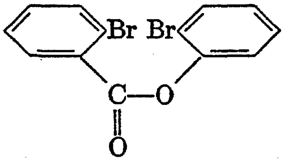

XXXVI

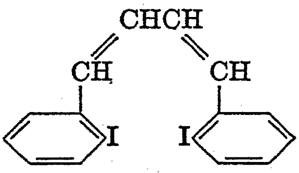
XXXVII


XXXVIII

XXXIX

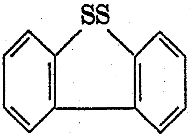
Cahn (32) was unable to effect an intramolecular Ullmann reaction of 2-bromophenyl 2-bromobenzoate (XL).

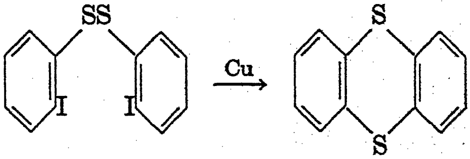

XL


XLI

The attempted intramolecular Ullmann reaction of 2,2′-diiododistyryl (XLI) was also unsuccessful (153).

A good yield of thianthrene (XLIc) was obtained as the product of the attempted synthesis of the cyclic disulfide XLIa from di-*o*-iodophenyl disulfide (XLIb) (13).

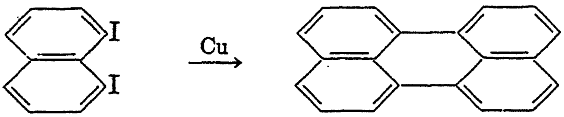

XLIa


XLIb

XLIc

D. Reactions of polyfunctional compounds

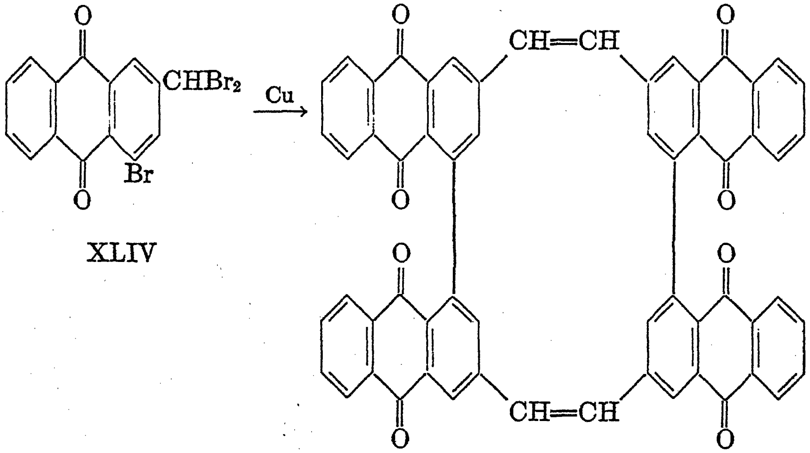
Cyclic products may also be obtained when polyhalogenated aromatic compounds are subjected to the conditions of the Ullmann reaction. The synthesis of perylene (XLIII) from 1,8-diiodonaphthalene (173a) is an example of such a reaction.



XLII
1,8-Diiodonaphthalene

XLIII
Perylene

Similarly, XLIV has been reported to form XLV (155).



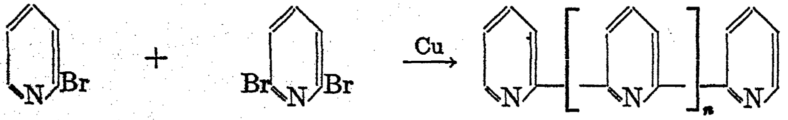
XLIV

XLV

Sircar and Majumdar (186) have reported that a tetraphenylene, presumably cyclic, is obtained when 4,4′-diiodobiphenyl is subjected to the conditions of the Ullmann reaction.

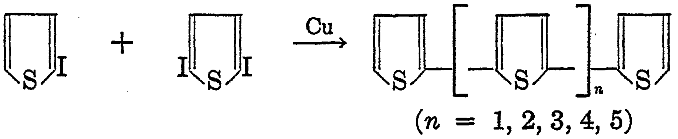
Attempts to prepare biphenylene (XXXIII) by reactions of the polyfunctional type were unsuccessful. When either 2-bromoiodobenzene (126) or 1,2-diiodobenzene (49) was heated with copper powder, only resins were obtained.

The reaction of a mixture of polyfunctional and monofunctional compounds may yield a mixture of low polymers. Thus, Burstall (28) obtained a mixture of di-, tri-, and tetra-pyridyls by the reaction of 2-bromopyridine and 2,6-dibromopyridine.

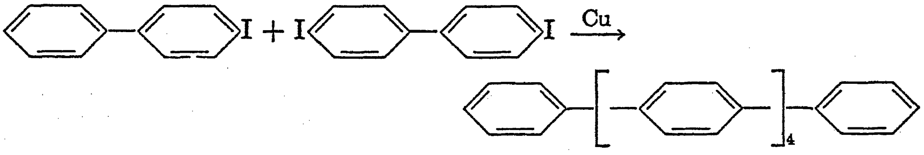


2-Bromopyridine 2,6-Dibromopyridine ($n = 0, 1, 2$)

The same technique was used by Steinkopf and his coworkers (201, 205) in the preparation of polythienyls.



Pummerer and Seligsberger (152) made use of the same principle in the preparation of sexiphenyl.



Sexiphenyl

III. EXPERIMENTAL CONDITIONS

The experimental technique employed in carrying out the Ullmann reaction is simple. In general, the aromatic halide is maintained at a suitable temperature in an open tube or flask while finely divided copper is added. In certain cases various special precautions must be observed.

A. Starting materials

As far as can be determined by an examination of the literature, the organic halides used in the Ullmann reaction in general need not be specially purified.

On the other hand, the nature of the copper used in the reaction has been the subject of considerable discussion and controversy. Commercial pulverized copper, known also as copper bronze, has long been one of the most commonly employed forms of the metal. Frequent reference is made to a particular brand of copper bronze known as "Naturkupfer C", which was obtainable from Bernhard Ullmann and Co. of Fürth (215, 216).

Freshly precipitated copper has also been used successfully (22). This is prepared by the treatment of zinc dust with copper sulfate solution, followed by washing with alcohol and ether and drying. However, Ullmann and Bielecki (219) and also Schreiner (178) observed that the mechanically pulverized copper bronze is preferable to the chemically precipitated or so-called molecular copper.

Commercial copper bronze contains traces of oil which is used during its preparation. This impurity is readily removed by washing with ligroin or ether, but Ullmann and Bielecki (219) observed that preliminary treatment is unnecessary when the copper is to be used in the biaryl synthesis. On the other hand, Kleiderer and Adams (95) recommend activation of copper bronze by treatment with iodine in acetone, followed by washing with hydrochloric acid and acetone and drying in a desiccator. They state that whereas ordinary copper bronze sometimes gives poor results in the Ullmann reaction, this simple treatment insured more uniform results and frequently made possible a more rapid reaction at a lower temperature.

Müller and Tietz observed (146) that when methyl 3,5-dichloro-4-iodobenzoate was treated with Naturkupfer C which had been reduced in hydrogen at 250–300°C., the hydrogen retained by the copper effected a partial reduction to methyl 3,5-dichlorobenzoate, with a consequent lowering in the yield of the desired biphenyl derivative. An improved yield was obtained when they used "Naturkupfer purified in nitrogen."

Lothrop (120, 121) found that cuprous oxide was effective in the synthesis of biphenylene from 2,2′-dihalogen biphenyls or 2,2′-biphenyleneiodonium iodide, while pure copper metal was without effect.

In the experience of the author (33a, 210a), Baker's precipitated copper powder has been found to be quite satisfactory for use without preliminary treatment. "Gattermann's precipitated copper powder" has been reported (155b) to be more active than the best copper bronze.

B. Proportions of reacting substances

A generous excess of copper is always used in the Ullmann reaction. Three times the theoretically required quantity is frequently employed, although two or three times that amount may be used for small batches when less reactive organic halides are being subjected to the reaction.

Since the reaction is often vigorously exothermic, only a small portion of the requisite amount of copper is placed in the reaction mixture at the outset, and the remainder of the metal is added in small portions during the course of the reaction.

C. Temperature

The temperature employed in the Ullmann reaction varies from 100° to 360°C., depending upon the reactivity of the aromatic halide.

In the case of any particular reaction, two temperatures are of importance: (*1*) the temperature at which reaction occurs at a reasonable and controllable rate; (*2*) the maximum temperature to which the reaction mixture may be subjected without causing the occurrence of undesirable side reactions or decomposition. For example, reactions of compounds containing the nitro group must not be carried out at a temperature exceeding 240°C.; otherwise reduction of the nitro groups by the copper may occur (61).

The desired temperature may usually be most conveniently maintained by immersing the reaction vessel in a heated metal bath. The use of two thermometers, one immersed in the metal bath and the other directly in the reaction mixture, is desirable. In the case of small-scale reactions, the latter thermometer may also be employed as a stirring rod.

The optimum reaction temperature is probably that at which the gradual addition of the copper suffices to maintain an observable yet readily controllable exothermic reaction. In the case of reactive halides, the evolution of heat may serve to keep the reaction mixture at a temperature 5° to 20°C. above that of the bath. Toward the completion of the reaction less heat is evolved, and the temperature of the bath must be increased. A temperature somewhat higher than the optimum reaction temperature is usually maintained for some time after all of the copper has been added and the observable reaction has subsided. Long-continued heating in this manner probably serves no useful purpose (92).

The reaction temperature of low-boiling compounds can sometimes be attained only by the use of a sealed tube or bomb (10, 36, 136, 167, 216).

In most cases the reaction may be carried on successfully over a considerable range of temperature, while in other cases the temperature control appears to be extremely critical (226).

D. Diluents

The violence of the reaction which occurs when very reactive halides are used in the Ullmann reaction may be moderated by the use of a diluent. The following organic substances have been used for this purpose: nitrobenzene, toluene, naphthalene, *p*-cymene, biphenyl, and anthracene. Sand has also been employed (61, 219), even when its use was probably superfluous (105). The chief purpose of sand in the reaction mixture in large-scale runs is to assist in breaking up and extracting the soluble material from the hard mass which is usually produced.

Substitution of a hydrogen atom for the aromatic halogen occurred when tetralin was used as a diluent (69, 111).

An aqueous solution of copper sulfate has been used as a diluent in the Ullmann reaction of certain halogenated aromatic sulfonates (13, 46).

E. Protective atmosphere

The Ullmann reaction is sometimes conducted in an atmosphere of carbon dioxide, hydrogen, or nitrogen. It is not evident under what conditions this precaution is of value, but in at least one reported case (70) a definite improvement in yield was observed when nitrogen was used to protect the reactants from the air. Further examples of the use of a protective atmosphere are to be found in tables 1 and 2.

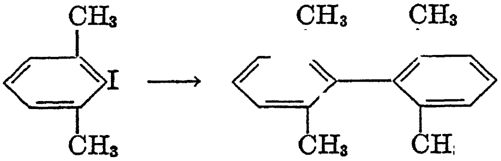
F. Agitation

As copper powder is added to the medium during the reaction, it rapidly settles to the bottom and must be stirred to expose the active surface of the metal. In the case of small-scale reactions employing 5 to 25 g. of material, a thermometer usually serves as a rod for intermittent manual stirring. In mechanical stirring of larger-scale batches, the paddle must be able to agitate the heavy sludge of spent copper which settles to the bottom of the vessel. In the case of reactions which must be carried out in a sealed tube or under a protective atmosphere, provision for rocking or rotating the vessel is advisable.

G. Isolation of products

The procedure used in isolating the products depends greatly upon the chemical nature of the substances obtained. The considerations which apply particularly to the unsymmetrical biaryls have been discussed in Section II B. The isolation procedure usually utilizes polar or reactive groups in the molecule.

Thus, products containing one or more carbalkoxyl groups are saponified and isolated as the corresponding carboxylic acids. In the preparation of biphenyl XLVII from 2,6-dimethyliodobenzene (XLVI) (33a), difficulty was experienced in separating the product from unchanged starting material. Treatment of the mixture with Raney nickel gave a readily separable mixture of the biphenyl and *m*-xylene.



XLVI XLVII

Further detailed procedures which are applicable to specific types of product are to be found in the literature cited in tables 1 and 2.

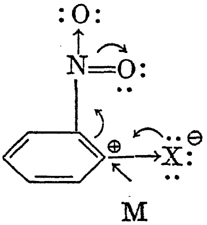
IV. MECHANISM

The mechanism of the Ullmann reaction is not known. However, by analogy with the reactions of other metals with organic halides, we might expect to find as intermediates either (*1*) free radicals, (*2*) organometallic compounds of moderate stability, or (*3*) metallic complexes of transitory existence.

The possibility of a free-radical mechanism has been suggested by Rapson and Shuttleworth (153a). They found that when iodobenzene was caused to react with copper in the presence of ethyl benzoate, the product after saponification contained 2- and 4-diphenylcarboxylic acids. It seemed probable that these products were the result of the formation by the iodobenzene and copper of a free phenyl radical which displaced a hydrogen atom from either the ortho- or the para-position of the ethyl benzoate. However, in all cases in which the Ullmann reaction has been carried out in the usual way, biaryl bond formation was observed to occur only at the carbon atom from which a reactive halogen atom had been displaced. Since the existence of a free-radical intermediate would be expected often to lead to a variety of products, it cannot be considered a plausible mechanism for the normal reaction. Because free radicals readily lead to polymerization, their occurrence as the result of a side reaction offers an explanation for the formation of polymers (201) or resinous materials which are sometimes obtained as by-products of biaryl formation.

The preparation and reactions of organocopper compounds have been studied by Gilman and Straley (63). They point out that although well-characterized RCu compounds are known to undergo coupling to yield RR compounds, it is not known whether an organocopper compound of this type is formed as an intermediate in the Ullmann reaction. An R_2Cu compound has also been suggested as a possible intermediate in the coupling reaction of RCu, but there is no experimental evidence for the existence of such a compound.

Any mechanism proposed for the Ullmann reaction must be in agreement with two facts: (*1*) the activity of a series of aromatic halides increases in the order $RCl < RBr < RI$; (*2*) activity is increased by $-T$ substituents in the ortho- and para-positions. The fact that these $-T$ substituents produce a residual plus charge on the ortho- and para-positions suggests that a nucleophilic attack by the metallic copper (M) at the activated position may be an initial and rate-determining step. The halogen atom (X) would contribute to the activation by the net result of a $-I$ and a $+T$ effect. The combination of these two



effects must be such that the polarization of the R—X bond increases in the order RCl < RBr < RI.

Further evidence for the participation in the reaction of an activated complex is the reaction referred to on page 144. In this case Rule and Smith (155a) observed that although iodobenzene (XXXI) does not react with copper below 220°C. and picryl chloride (XXX) reacts explosively at 135°C., equimolar quantities of the two react in nitrobenzene solution at 160–165°C. to give an 85 per cent yield of the unsymmetrical biphenyl XXXII. They conclude that, "In the presence of picryl chloride the relatively nonreactive iodobenzene undergoes some special form of activation, permitting it to enter into reaction at a temperature much below that at which it would ordinarily do so." In terms of activated complex formation, the copper–picryl chloride complex reacts preferentially with iodobenzene. Rule and Smith suggest that steric hindrance of the ordinarily more reactive halogen atom of picryl chloride is the controlling factor in this preferential reaction.

We may conclude that a possible mechanism for the reaction consists in two consecutive steps: *(1)* A nucleophilic reaction of copper with the aromatic halide to form an activated complex at the metal surface. The occurrence of this step is governed largely by the susceptibility of the aromatic halide to nucleophilic attack. *(2)* The reaction of the activated complex with a second molecule of aromatic halide to form a biaryl molecule plus copper halide. This reaction is governed to a greater extent by steric factors. A more detailed knowledge of the mechanism must await further experimentation.

V. TABLES

Symmetrical biaryls which have been prepared by the Ullmann reaction are listed in table 1. The compounds have been arranged, with a few exceptions, according to the familiar Beilstein system.

Unsymmetrical biaryls are listed in table 2. In cases where non-equimolar amounts of the reactants were used the yield of unsymmetrical product was calculated on the basis of the component present in lower molar concentration.

A number of examples of unsuccessful attempts to use the Ullmann reaction are presented in condensed form in table 3. This table is perhaps less complete than table 1 or 2.

The data were obtained by a systematic search of the literature up to and including the 1944 *Chemical Abstracts*.

TABLE 1

Symmetrical biaryls (RR) prepared by the reaction $2RX \xrightarrow{Cu} RR$

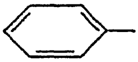
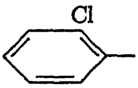
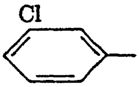
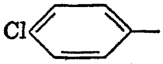
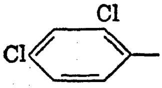
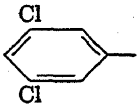
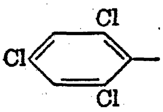
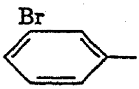
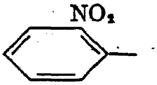
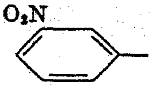
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	230	Sealed tube	82	(216)
	I	260			(227)
		150	Slow reaction		(132)
	Br	204	Slow reaction		(132)
	I	250		96	(226)
		250		67	(216)
		230	Slow reaction		(132)
	I	200–250		82	(216)
		226–227	Slow reaction		(132)
	I	200–270		53	(216)
	I				(230)
	I	220–230		52.5	(216)
	I			Poor	(216)
	Cl	210–245	With dry sand	60	(219)
		215–225	With dry sand	52–61	(61)
		240–245			(105, 106, 161)
	Br	230–240			(128)
		200–220		75	(219)
		185	Vigorous reaction		(132)
	I	134	Vigorous reaction		(132)
	X?	230			(157)
	I	210–225		26–52	(46, 161, 219)
		230	Moderate reaction		(132)
	Br	257	Slow reaction		(132)

TABLE 1—*Continued*

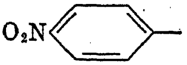
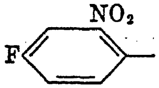
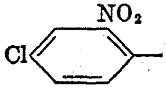
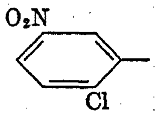
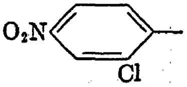
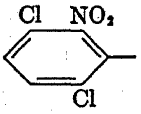
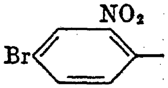
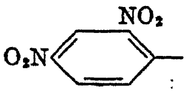
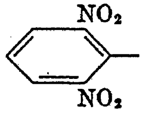
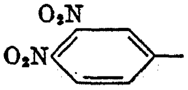
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	220–235		52	(219)
		287	Slow reaction		(132)
	Br	255	Slow reaction		(132)
	Br	240–250		46	(184)
	Cl	240–255		42	(219)
		240–245			(106)
	I	138	Rapid reaction		(132)
	I				(37a)
	I				(37a)
	I				(244)
	Br	190–225		65	(105, 219)
		210			(106)
	Cl		Boil in $C_6H_5NO_2$	60	(106, 219, 227)
		295	Violent reaction		(132)
	Br	157	Rapid reaction		(132)
			Boil in $C_6H_5NO_2$	65	(219)
	Cl		Boil in $C_6H_5NO_2$		(20)
	I	230–250		47	(219, 36a)

TABLE 1—*Continued*

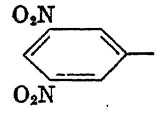
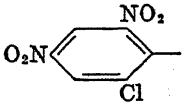
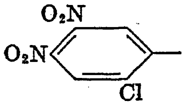
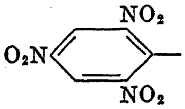
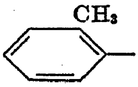
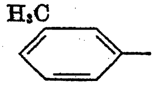
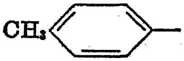
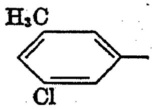
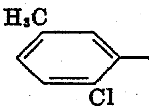
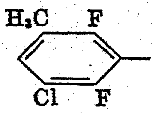
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	270		15	(36)
	I	240		46	(37a)
	I		Boil in $C_6H_5NO_2$	41	(37a)
	Cl		Boil in $C_6H_5NO_2$	55	(219)
		160–180	Boil in $C_6H_5NO_2$	58	(229a)
			Boil in $C_6H_5CH_3$ or $C_6H_5NO_2$		(194)
	I	230	Sealed tube	63	(90, 92, 106, 215)
		207	Slow reaction		(132)
	I	205–240		35	(216)
		204	Slow reaction		(132)
	I	210–260		54	(114, 216, 225)
		260		60	(140)
		211.5	Slow reaction		(132)
	I	250–290			(137)
	I				(207)
	I	200		34	(95)

TABLE 1—*Continued*

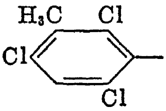
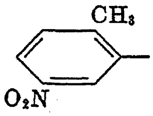
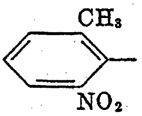
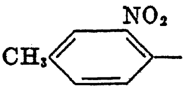
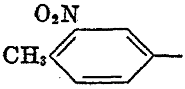
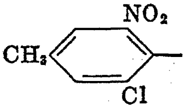
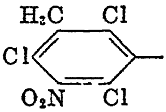
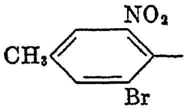
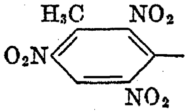
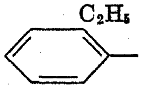
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		per cent	
	I	235–250			(237)
	I	220–270		22 Poor	(151) (37, 128, 132)
	I	180–235		80	(121, 240) (89, 99, 132, 139, 157, 233)
	Cl				(89, 221)
	Cl	260			(128)
	I		Boil in $C_6H_5NO_2$	39	(147)
		240–260			(48)
	Cl	240	Slow reaction		(132)
	I	230–250			(29)
	I	235–250			(236)
	I		In solution		(244)
	Br	160–183	In $C_6H_5NO_2$	70	(18)
	I	245–255			(131)
		226	Slow reaction		(132)

TABLE 1—*Continued*

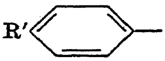
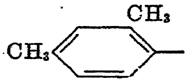
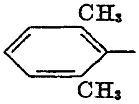
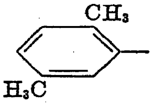
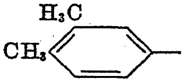
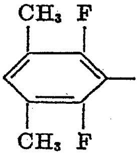
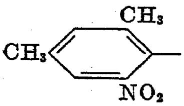
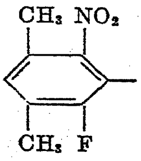
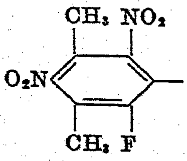
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
					
R′ = C_2H_5	I			50	(178)
R′ = $(CH_3)_2CH$	I				(178)
R′ = *n*-C_4H_9	I	300			(19)
R′ = $C_2H_5(CH_3)CH$	I	300			(19)
R′ = $(CH_3)_3C$	I				(178)
R′ = $C_2H_5(CH_3)_2C$	I	300		40	(19)
	I	230–260		86	(216)
		300	Sealed tube	60	(167)
		232	Slow reaction		(132)
	I	240–265		21	(33a)
	I	230–260		46	(216)
		229	Slow reaction		(132)
	I	220–268		23	(44)
	I	200		60	(95)
	I				(233)
	Br	200	In $C_6H_5NO_2$	75	(93)
	Br	190	In $C_6H_5NO_2$	69	(93)

TABLE 1—*Continued*

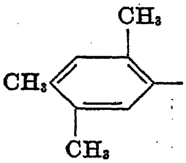
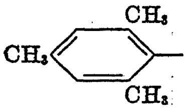
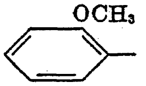
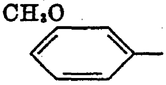
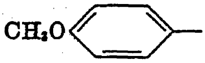
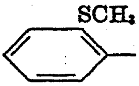
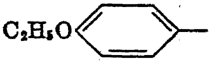
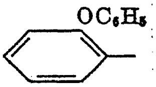
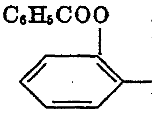
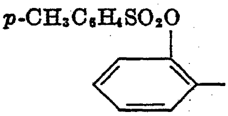
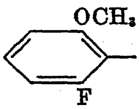
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		per cent	
	I	230–250		50	(216)
	I	260–270		Poor	(145, 216)
	I	210–260 213	 Rapid reaction	88	(216) (132) (225)
	I	240	Moderate reaction		(132) (225)
	I	230–240 225	 Moderate reaction	85	(216) (132)
	I	200–250		80	(24)
	I	230–240		75	(216)
	I	200–220			(111)
	I	215–260		40	(216)
	I	260			(216)
	I	180–200		65	(14)

TABLE 1—*Continued*

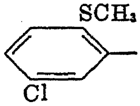
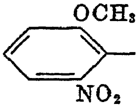
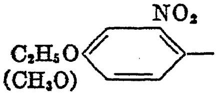
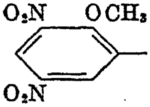
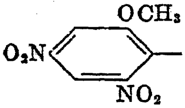
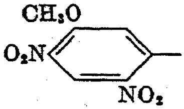
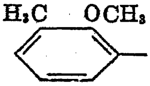
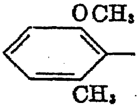
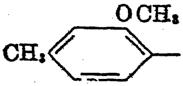
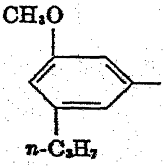
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	200–280		25	(23)
	Cl	180–210	In $C_6H_5NO_2$	70	(2)
	I	240 185–220 130–170			(225) (22) (71)
	I		Boil in $C_6H_5NO_2$	12	(37a)
	Cl		Boil in $C_6H_5NO_2$		(227)
	Cl	235		Poor	(226)
	I	240			(209)
	I				(209)
	I				(209)
	I	270			(208)

TABLE 1—*Continued*

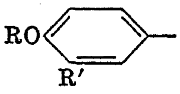
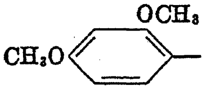
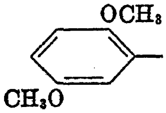
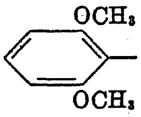
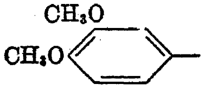
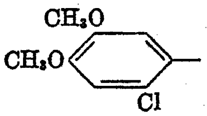
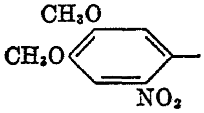
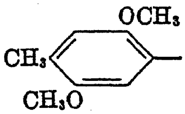
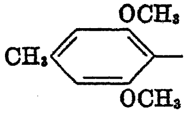
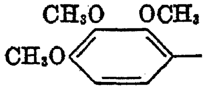
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		per cent	
	I		Cu not specified		(26)
	I	260	CO_2 atmosphere	75	(193)
	I	215–260		93	(216)
	I	170–210 170–190		85–90	(229) (185)
	I I	260–270 260	CO_2 atmosphere CO_2 atmosphere	90	(193) (181)
	I	270–280	CO_2 atmosphere	20	(181)
	Br	210–240		70	(74)
	I				(239a)
	I				(148a)
	I				(56)

TABLE 1—*Continued*

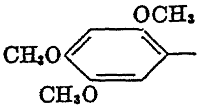
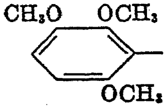
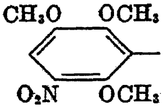
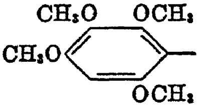
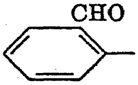
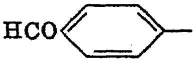
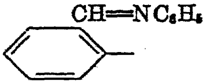
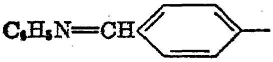
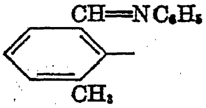
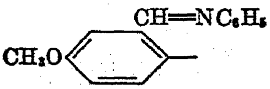
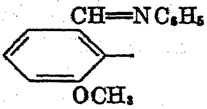
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	Br	250–270			(59)
	I	185–215	N_2 atmosphere	76	(63a)
	I	210–230		15	(63a)
	I	230			(55)
	I	200–220		70	(153)
		200–220	Isolated as the dioxime		(133)
	Cl	208	Slow reaction		(132)
	I		Isolated as the phenylhydrazone		(216)
	I	160–180	H_2 atmosphere	74	(134, 234)
	I	260			(216)
	I	160	H_2 atmosphere		(135)
		210	H_2 atmosphere		(134)
	I	160–180	H_2 atmosphere		(135)
	I	200	H_2 atmosphere		(134)

TABLE 1—*Continued*

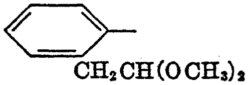
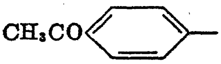
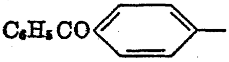
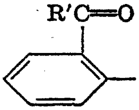
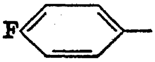
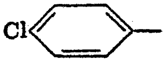
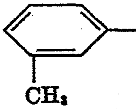
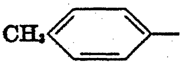
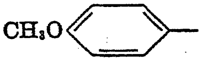
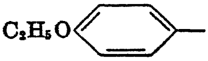
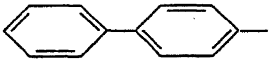
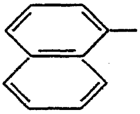
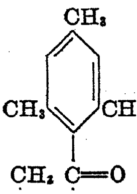
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	235–260		82	(234)
	I	235–260		25	(60)
	I	250		55	(216)
	Br				
R′ = 		200		30	(9)
R′ = 				0	(9)
R′ = 		250		38	(9)
R′ = 		200		68	(7)
R′ = 		200		50	(7)
R′ = 		250		57	(9)
R′ = 		200		22	(9)
R′ = 		200		66	(9)
	Br	200		65	(58)

TABLE 1—*Continued*

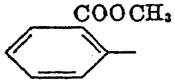
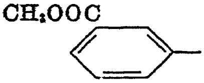
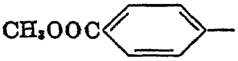
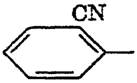
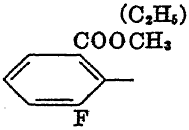
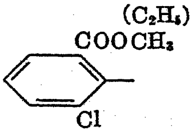
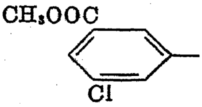
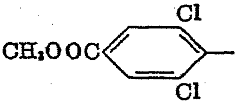
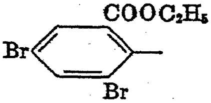
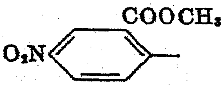
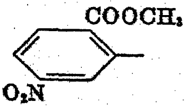
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	250–260		82	(216)
	I	260		60	(216)
	I	220–260		70	(211, 212, 216)
	I	275			(68)
	I	180–240		57	(196)
	I	180–240			(39)
	I	265–270		25	(137)
	I	280	Sealed tube		(146)
	I				(243)
	Br	180–220			(219)
	I	200		75	(149; *cf.* 37)

TABLE 1—*Continued*

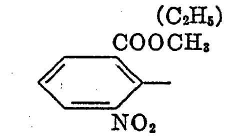
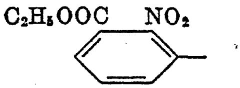
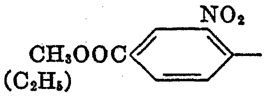
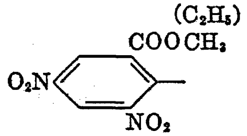
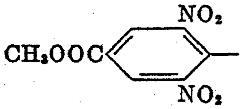
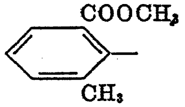
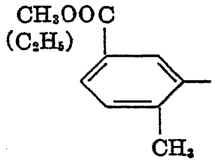
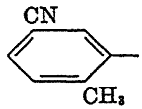
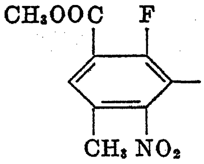
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	Cl	210–235		80–85	(89)
	Br or I	165–175		83	(82, 89, 101)
	Br	180			(31)
	Br	170–185		81	(219)
	I		Boil in $C_6H_5NO_2$	69	(180)
	Cl	140–160		62	(39a, 102, 111, 217)
	Cl		Boil in $C_6H_5NO_2$	54	(219)
	I	220 270		Poor	(133) (15)
	I	260		55–65	(92)
	I	260		Poor	(92)
	Br	220–225		65	(94)

TABLE 1—*Continued*

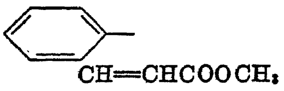
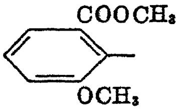
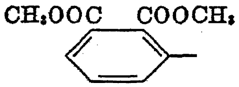
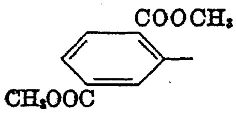
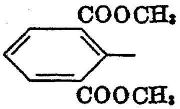
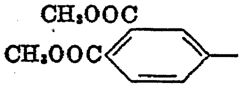
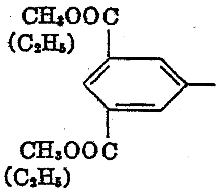
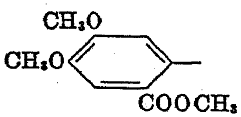
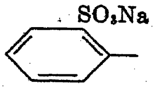
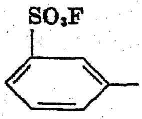
R	X	TEMPERATURE °C.	REMARKS	YIELD per cent	REFERENCE
	I	200–300		Poor	(234)
	I	205–215		70	(91, 196)
	I	240–260			(87, 88)
	I	260–310		70	(92)
	I	140–200			(133, 140)
	I	260			(88)
	I	220–250			(30)
	Br	255–260			(96)
	I		Boil in aqueous solution with $CuSO_4$	75	(13)
	I	200–230		54	(200)

TABLE 1—*Continued*

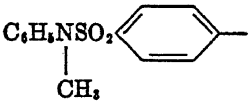
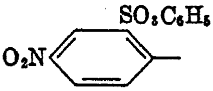
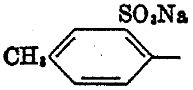
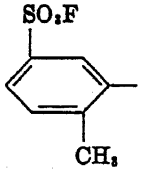
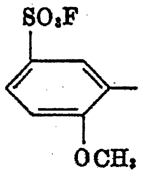
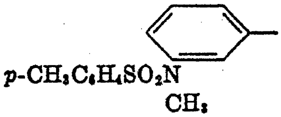
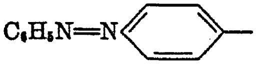
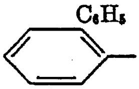
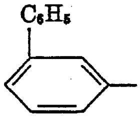
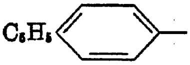
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I	210–240			(216)
	I Cl	205 275		75 Poor	(112) (112)
	I		Boil in aqueous solution with $CuSO_4$		(13)
	I	220		41	(200)
	I	220			(200)
	I	212–230			(216)
	I	260			(216)
	I	255–310		72	(10, 21)
	I				(21)
	I Br	220–270 295–300		82.5 Trace	(21, 216; *cf.* 62) (21)

TABLE 1—*Continued*

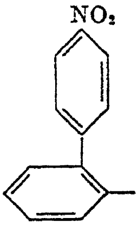
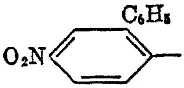
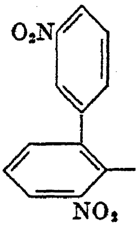
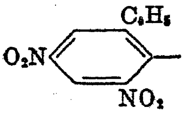
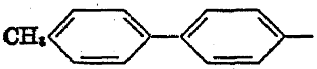
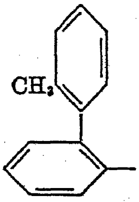
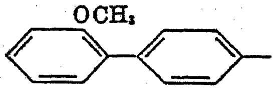
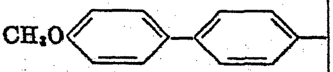
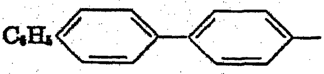
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I				(158)
	I				(159)
	I	190–195			(160)
	Cl	215		Small	(21a)
	I	220		60–70	(152)
	I	265–285		70	(156)
	I	280	N_2 atmosphere	70	(70)
	I	280	N_2 atmosphere		(70)
	I	280–300		Poor	(150)

TABLE 1—*Continued*

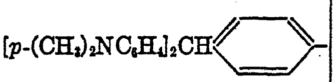
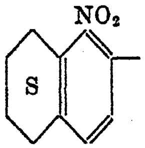
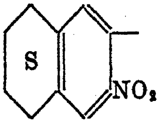
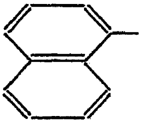
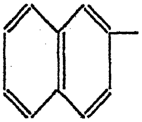
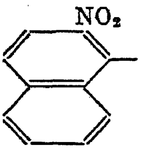
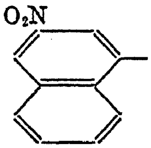
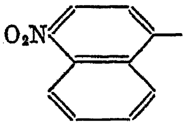
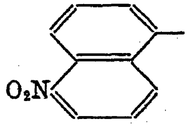
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	Br	210–220	H_2 atmosphere		(50)
	I	135–190		20	(45)
	I	110–140		80	(45)
	I	260–285		74–92	(25, 219)
	Br	280–285	I_2 added	50	(177)
	I	230–260		67.5	(216)
	I	120–130		70	(40, 45)
	I			20	(40, 45)
	I	220–230	In naphthalene	50	(40, 177)
	I	220–230		60	(174)

TABLE 1—*Continued*

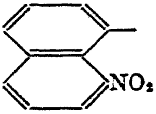
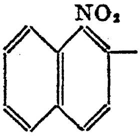
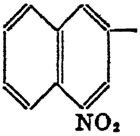
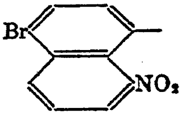
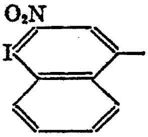
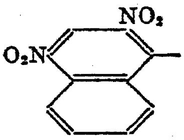
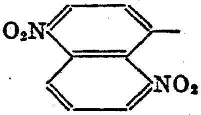
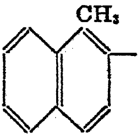
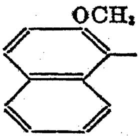
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	I		Boil in $C_6H_5NO_2$	43	(73)
	I		Boil in $C_6H_5NO_2$	50	(40, 231)
	I		Boil in $C_6H_5NO_2$	30	(72)
	I		Boil in $C_6H_5NO_2$	29	(73)
	I		In $C_6H_5NO_2$		(72)
	Cl		Boil in $C_6H_5NO_2$		(40, 154)
	I		Boil in $C_6H_5NO_2$	36	(73)
	I	220–260			(175; *cf.* 235)
	I	200–210	I_2 added	30	(41)

TABLE 1—*Continued*

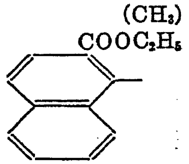
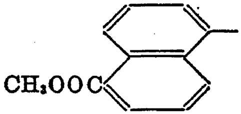
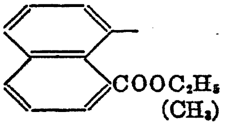
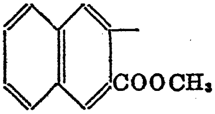
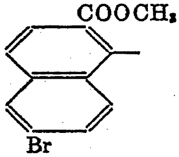
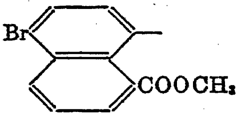
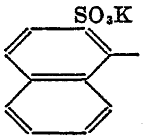
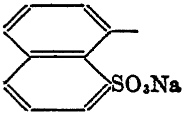
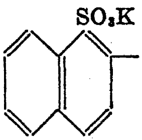
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	Br	190		60	(124)
	Cl	300			(86)
	I	220–240	CO_2 atmosphere	75	(182)
	Cl	290–300	I_2 added		(85, 86, 104)
	Br	190–200		90	(124)
	Br	160–170		41	(155a)
	Br	210–220		55	(155a)
	I		Boil in aqueous solution with $CuSO_4$		(13)
	I		In aqueous solution with $CuSO_4$		(46)
	I or Br		In aqueous solution with $CuSO_4$		(46)

TABLE 1—*Continued*

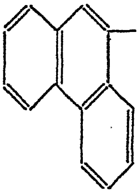
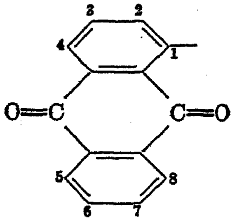
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	Br	330–350		10	(246; *cf.* 8)
	Cl		Boil in $C_6H_5NO_2$	70–80	(142, 224)
	I	210–275	CO_2 atmosphere	20	(168)
	—SCN or —SS—	220–240	In anthracene		(97)
	X				(12)
Substituted 1,1'-bianthraquinolyls:					
3-Cl	Cl		Boil in $C_6H_5NO_2$		(53)
4-Cl	Cl		Boil in $C_6H_5NO_2$		(53)
2-Br	Br	200–210	In $C_6H_5NO_2$	68	(179)
3-Br	Br		Boil in $C_6H_5NO_2$	65	(220)
2-NO_2	Cl		In $C_6H_5NO_2$		(98)
2-substituted	X				(187, 188)
2-CH_3	I	210–290		30–50	(103, 162)
	Cl	225–230	Boil in $C_{10}H_8$		(165)
	—SS—				(97)
	X				(11)
3-CH_3	Br		Boil in $C_6H_5NO_2$	50–70	(155)
4-CH_3	Cl		Boil in $C_6H_5NO_2$	70	(224)
2-C_2H_5	I	240	CO_2 atmosphere	50	(173)
2-*n*-C_3H_7	I	200	CO_2 atmosphere		(173)
2-$(CH_3)_2CH$	I	200	CO_2 atmosphere	75	(173)
2-CH_3, 4-CH_3	I	210–250	CO_2 atmosphere	35	(163)
2-OH	—SCN or —SS—				(97)
2-OCH_3	I	360		20	(17)
4-OCH_3	Br or Cl		Boil in $C_6H_5NO_2$		(52)

TABLE 1—*Continued*

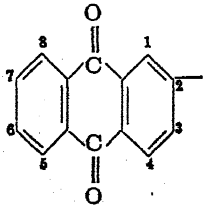
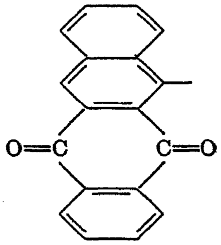
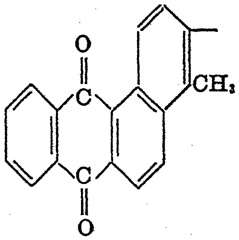
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
2-C_6H_5COO	Br		Boil in $C_{10}H_8$		(69)
3-OCH_3, 4-OCH_3	I	310	CO_2 atmosphere	53	(181)
2-CHO	Cl				(12a, 83)
2-CHO, 6- or 7-Cl	Cl		In a solvent		(12a)
2-$COOC_2H_5$	Cl		Boil in $C_6H_5NO_2$	75	(169)
4-$COOCH_3$	Cl	250–300		40	(195)
4-C_6H_5COO, 3-CH_3O	Br		Boil in $C_6H_5NO_2$ or $C_{10}H_8$		(148)
2-$NCHC_6H_5$	Cl	220–240			(166)
2-$NCHC_6H_5$, 3-Br	Br	220–260	In $C_{10}H_8$	51	(218, 223)
2-$NHCOOC_2H_5$	Cl	135–175			(189)
2-$N(COR)_2$	X				(77, 78, 80, 81)
	I	230–330	CO_2 atmosphere	30	(171)
Substituted 2,2'-bianthraquinolyls:	I	360		2	(17)
1-OCH_3	I		Boil in $C_{10}H_8$		(69)
1-C_6H_5COO	Br		Boil in $C_6H_5NO_2$	58	(170)
1-CN					
	Cl	220–230	In $C_6H_5NO_2$		(232; *cf.* 76)
	I	200–360	CO_2 atmosphere		(172)

TABLE 1—*Continued*

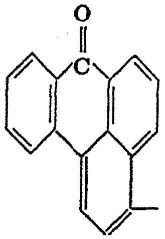
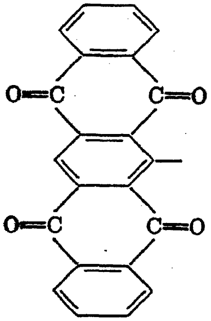
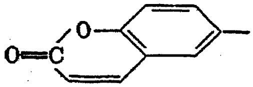
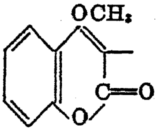
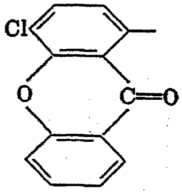
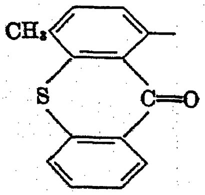
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		per cent	
	Br	280–300			(122)
	Br or Cl		Boil in $C_6H_5NO_2$		(47)
	I				(183)
	Br	210		30	(73a)
	Cl		Boil in $C_{10}H_8$		(51)
	Cl		Boil in $C_{10}H_8$	46	(222)

TABLE 1—*Continued*

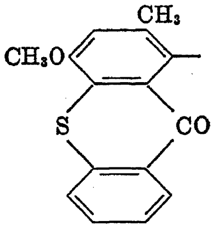
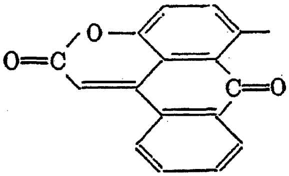
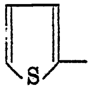
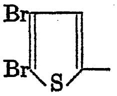
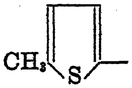
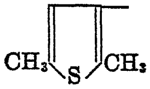
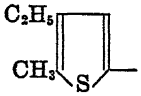
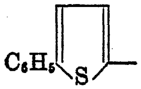
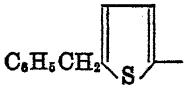
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		*per cent*	
	Cl		Boil in $C_{10}H_8$	57	(222)
	Cl		Boil in $C_6H_5NO_2$	40	(143)
	I	190–210			(201)
	I	240			(197a)
	I	150–220		75–81	(202)
	I	220–250		47	(198, 204)
	I	210–245		20	(203)
	I				(201)
	I	185–210		14	(199)

TABLE 1—*Concluded*

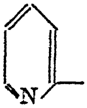
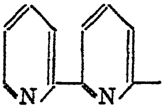
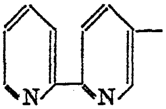
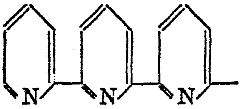
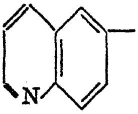
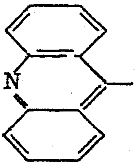
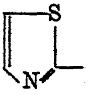
R	X	TEMPERATURE	REMARKS	YIELD	REFERENCE
		°C.		per cent	
	I Br 	180 180 230	In *p*-cymene In *p*-cymene In $C_6H_5C_6H_5$	20 60 70	(238) (238) (28)
	Br		Boil in $C_6H_5C_6H_5$	30	(28)
	Br		Boil in $C_6H_5C_6H_5$		(28)
	Br			30	(28)
	I	220–340	Violent reaction		(216)
	Cl	140	In absence of air	60	(108)
	Br	170–180	In *p*-cymene		(57)

TABLE 2

Unsymmetrical biaryls prepared by the reaction RX + R′X′ $\xrightarrow{Cu}$ RR′

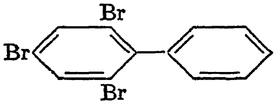
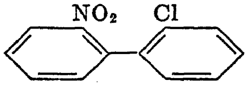
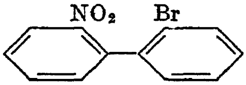
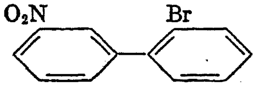
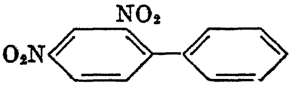
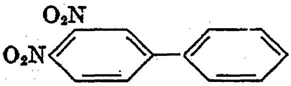
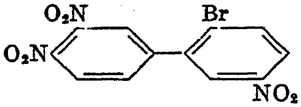
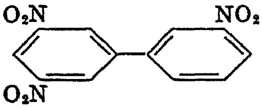
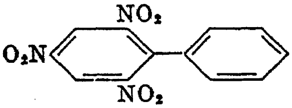
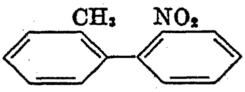
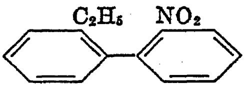
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	I	I	200		40	(35)
	I	I	200–225		25	(127)
	I or Br	I				(126, 127)
	I	I	250		10	(34)
	Cl	I	210–230		20	(66)
	I	I	280	Sealed tube	15	(36)
	I	I				(36b)
	I	I	270		Poor	(36)
	Cl	I	160–165		85	(66, 155a)
	I	I	220–260		25–37	(156, 184a)
	I	Cl	260–270			(131)

TABLE 2—*Continued*

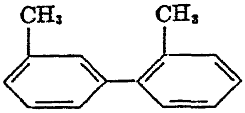
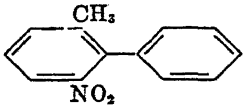
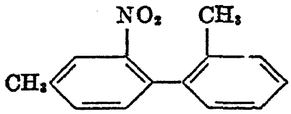
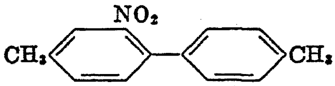
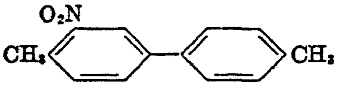
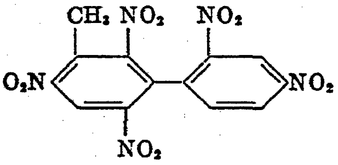
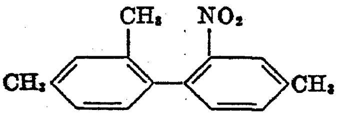
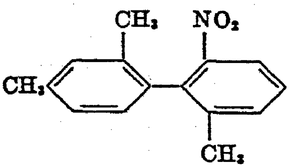
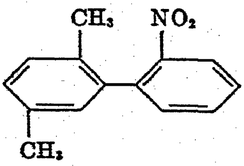
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	I	I	230–240	In a bomb		(136)
	Br	I	175–230		30	(156)
	I	I	260			(128)
	Br	I	225–260			(123)
	I	I	230–250			(123)
	Br	Cl	160–165	In $C_6H_5NO_2$	40	(197)
	X	X				(116)
	I	I	240–250			(131)
	I	I	230–260			(129)

TABLE 2—*Continued*

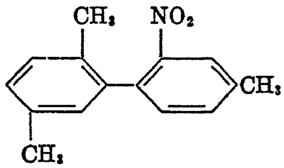
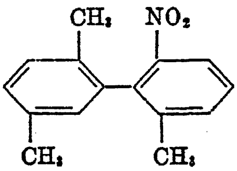
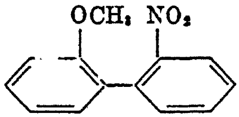
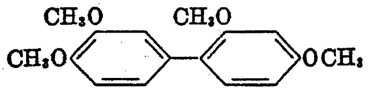
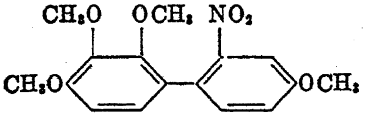
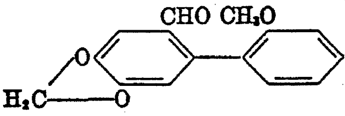
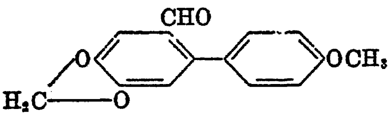
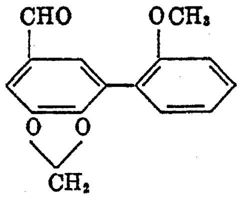
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	I	I				(117)
	X	X				(118)
	I	I				(125)
	I	I	260	CO_2 atmosphere	15	(193)
	I	Br	230–245		30	(210a)
	Br	I			20	(214)
	Br	I			20	(217)
	Br	I	220–230		15	(214)

TABLE 2—*Continued*

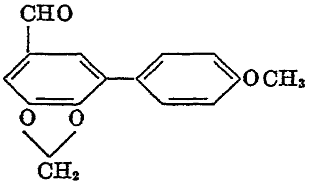
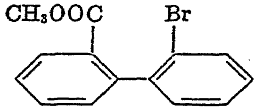
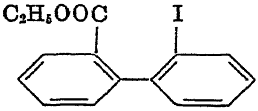
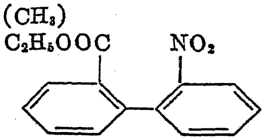
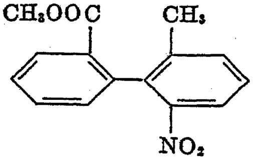
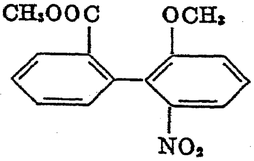
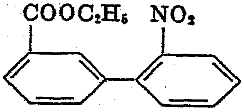
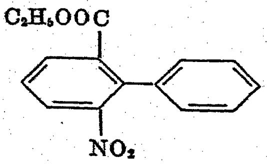
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	Br	I	220–250		5	(214)
	I	I	260–290		14	(141)
	I	I	280–300		35	(184a)
	I	I	230–270			(15)
	Br	I	250–285		20–68	(156, 184a)
	I	Br	270–300		27	(206)
	I	Cl	255–280		5	(2)
	I	Br	215–250		31	(1)
	Br	I	200–250			(156)

TABLE 2—*Continued*

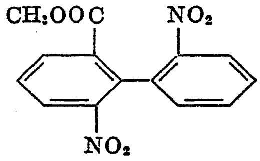
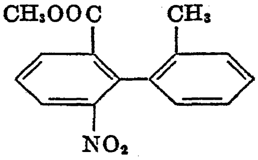
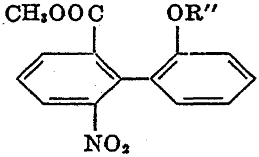
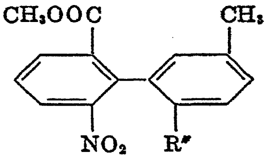
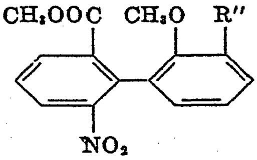
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	Br	I	220–225		30	(4)
	Br	I	225–230		15	(4)
						
R″ = CH_3	Br	I	240–250		18.4	(113, 207)
R″ = C_2H_5	Br	I	240–250		16.5	(113)
R″ = n-C_3H_7	Br	I	250–260		5.7	(113)
R″ = n-C_4H_9	Br	I	210–220		10.6	(113)
R″ = n-C_5H_{11}	Br	I	220–230		11.0	(113)
						
R″ = F	Br	I			16	(207)
R″ = Cl	Br	I			11	(207)
R″ = Br	Br	I			10	(207)
						
R″ = Cl	Br	I	220–250		7.9	(38)
R″ = Br	Br	I	220–250		3.7	(38)
R″ = NO_2	Br	I	220–250		15.5	(38)
R″ = CH_3	Br	I	240–280		18.7	(38)
R″ = OCH_3	Br	I	220–250		17.2	(38)

TABLE 2—*Continued*

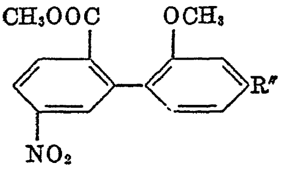
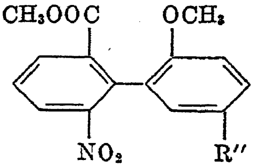
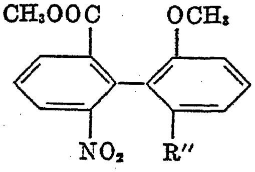
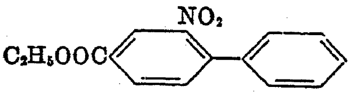
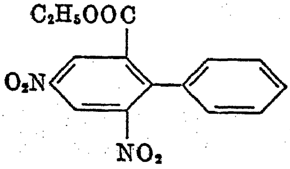
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
						
$R'' = Cl$	Br	I	205–215		10	(67)
$R'' = Br$	Br	I	240–250		10	(67)
$R'' = NO_2$	Br	I	230–240		10	(67)
$R'' = CH_3$	Br	I	205–215		10	(67)
$R'' = OCH_3$	Br	I	240–290		10	(67)
						
$R'' = Cl$	Br	I	240		30	(242)
$R'' = Br$	Br	I	200–215		30	(242)
$R'' = NO_2$	Br	I	230–240		10	(242)
$R'' = CH_3$	Br	I	240–260		15	(242)
$R'' = OCH_3$	Br	I	260–300		30	(241)
						
$R'' = F$	Br	I	210–240		1	(228)
$R'' = OCH_3$	I	I	240–300		12	(228)
	Br	I	235–245			(65)
	I	Cl	240		70	(109)

TABLE 2—*Continued*

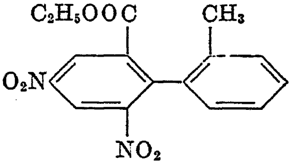
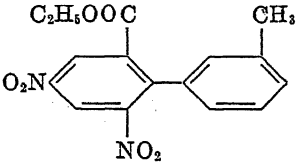
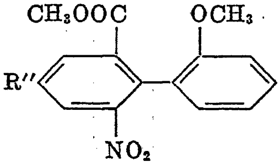
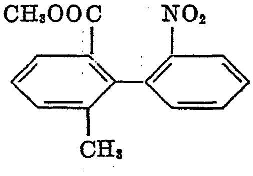
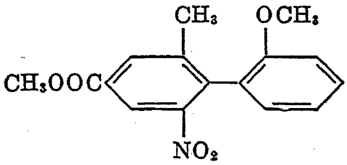
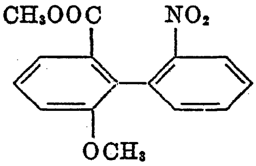
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
(*l*-menthyl) 	Cl	I	220		50	(109)
	Cl	I	230		90	(109)
						
R″ = Cl	I	I	210–230		45	(5)
R″ = Br	I	I	210–230		27	(5)
R″ = NO_2	I	I	215	In $C_6H_5NO_2$	23	(5)
R″ = CH_3	I	I	215	In $C_6H_5NO_2$	5	(5)
	I	I	230–250		50	(4)
	Br	I	240–270		28	(6, 213)
	I	I	200			(2)

TABLE 2—*Continued*

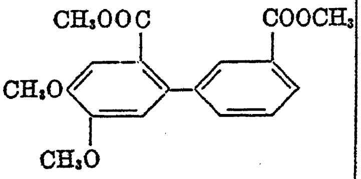
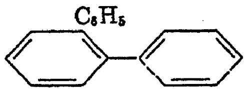
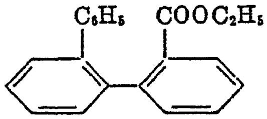
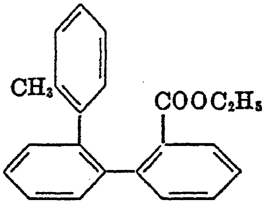
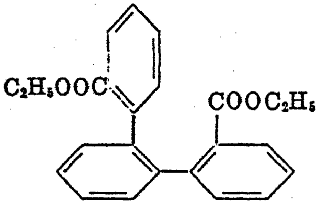
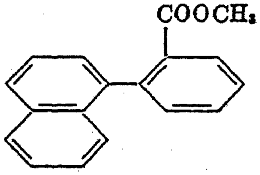
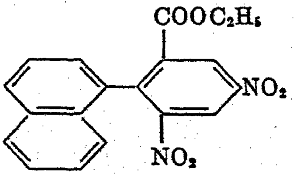
RR'	X	X'	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	Br	I	255–260		60	(96)
	I	I	240	Sealed tube	50	(10)
	I	Br	275			(156)
	I	Br	255–290		2	(156)
	I	I	280–320		5	(184a)
	I	I	280		30	(10d)
	I	Cl	210		70	(110)

TABLE 2—*Continued*

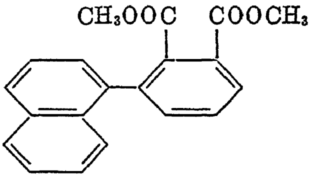
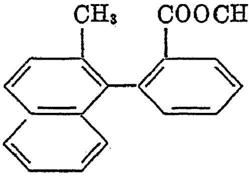
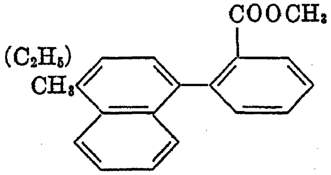
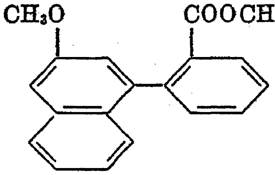
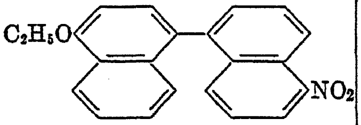
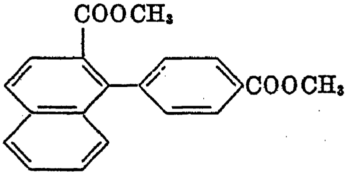
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	I	I		Boiling $C_6H_5NO_2$ bath	36	(10b)
	I	Br	255–260		10	(6a)
	I	I	180–190			(10c)
	I	I	180–190		10	(10a, 10d)
	I	I	180–210		26	(10b)
	I	I	220	CO_2 atmosphere		(210)
	Br	I	200–265		20	(10b)

TABLE 2—*Continued*

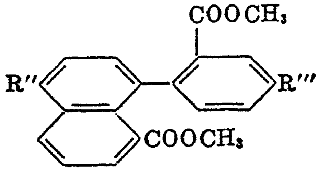
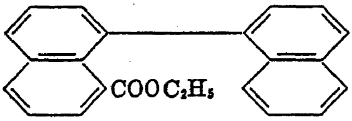
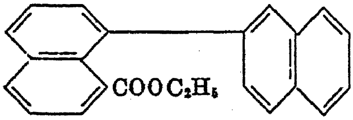
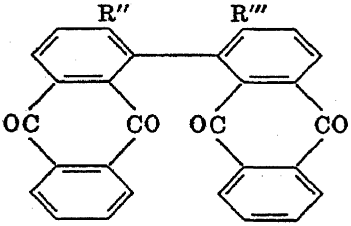
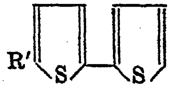
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
						
$R'' = H$; $R''' = H$	Br	Br or I	180–195		41–75	(155a, 155b)
$R'' = H$; $R''' = Br$	Br	I	180		60	(155a)
$R'' = H$; $R''' = NO_2$	Br	Br or I	180		33–41	(155a)
$R'' = Br$; $R''' = H$	Br	I	180		49	(155a)
$R'' = Br$; $R''' = Br$	Br	I	180		49	(155a)
$R'' = NO_2$; $R''' = H$	Br	I	180		49	(155a)
	Cl	Br	290		10	(138)
	Cl	Br	290		10	(138)
						
$R'' = CH_3CONH$; $R''' = H$	X	X				(144, 190)
$R'' = C_6H_5CHN$; $R''' = CH_3$	Cl	Cl	225–230	In $C_{10}H_8$	10	(165)
$R'' = C_6H_4(CO)_2N$; $R''' = CH_3$	Cl	Cl		Boil in $C_{10}H_8$		(79)
						
$R' = CH_3$	I	I	180–200		40	(201)
$R' = C_6H_5$	I	I	190–218		Poor	(201)

TABLE 2—*Concluded*

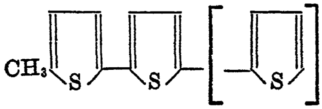
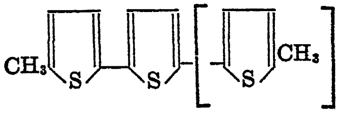
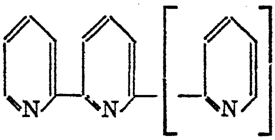
RR′	X	X′	TEMPERATURE	REMARKS	YIELD	REFERENCE
			°C.		*per cent*	
	I	I	200			(201)
	I	I	200			(201)
	Br	Br		In $C_6H_5C_6H_5$	5	(28)

TABLE 3

Unsuccessful reactions

A. Attempted syntheses of symmetrical biaryls

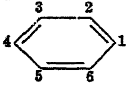
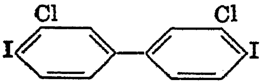
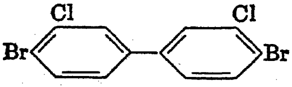
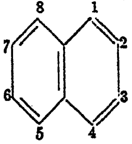
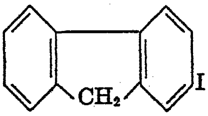
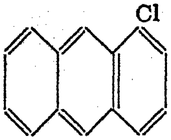
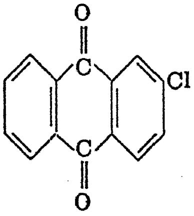
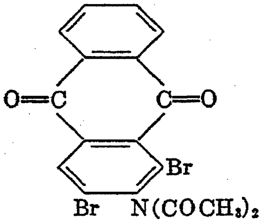
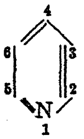
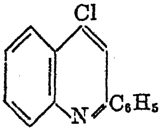
REACTANT	REFERENCE
	
Derivatives of benzene:	
1-Cl, 3,4-di-NO_2	(216)
1-Cl, 2-Cl, 4,6-di-NO_2	(227)
1-Cl, 2-NO_2, 3-CH_3	(64)
1-I, 4-$C(CH_3)_2C_2H_5$	(178)
1-I, 3-Br, 5-CH_3	(137)
1-I, 2,4,6-tri-CH_3, 3-NO_2	(145)
1-I, "phenols"	(216)
1-I, 3-OH, 6-CH_3	(151)
1-I, 3-$OCOC_6H_5$, 6-CH_3	(151)
1-Cl, 2,4,6-tri-NO_2, 3-OCH_3	(176)
1-Br, 2-F, 3,5-di-CH_3, 6-OCH_3	(14)
1-Cl, 2,4,6-tri-NO_2, 3,5-di-OCH_3	(176)
1-I, 2-$CH(OC_2H_5)_2$	(153)
1-I, 2-$CH{=}CHCOOCH_3$	(133)
1-I, 3-Br, 5-$COOCH_3$	(137)
1-I, 3-NO_2, 5-$COOC_2H_5$	(137)
1-Br, 2-NO_2, 4-$N(CH_3)_2$	(16)
1-I, 4-SO_3H	(216)
1-I, 4-$SO_3Ca_{1/2}$	(216)
1-I, 4-SO_2NH_2	(216)
1-I, 4-$SO_2NHC_6H_5$	(216)
1-I, 3 or 4-NH_2	(216)
1-I, 4-$NHCOCH_3$	(216)
1-I, 4-$NHCH_3$	(216)
	(33)
	(33)

TABLE 3—*Continued*

REACTANT	REFERENCE
	
Derivatives of naphthalene:	
2-I, 3-NO_2	(72)
1-I, 2,4-di-Br, 8-NO_2	(73)
1-Cl, 2,4,5-tri-NO_2	(154)
2-I, 3-CH_3, 6,7-di-OCH_3	(3)
2-Br, 3-CH_3, 1,4-di-$OCOCH_3$	(3)
2-Br, 3-CH_3, 1,4-di-OCH_3	(3)
1-Br, 2-SO_3Na	(46)
1-Cl, 4-SO_3Na	(46)
1-I, 5-SO_3Na	(46)
2-I, 6-SO_3Na	(46)
1-I, 2-SO_3Na	(46)
1-I, 4-SO_3Na	(46)
Penta- and hexabromo derivatives of 2,7,2′,7′-tetrahydroxy-1,1′-binaphthyl	(75)
	(100)
	(164)
	(224)
	(162)

REACTANT	REFERENCE
	
Derivatives of pyridine:	
2-Cl	(238)
2,5-di-Br	(239)
2,6-di-Br	(239)
2,3,5-tri-Br	(239)
2-Br, 6-CH_3	(239)
2-Br, 6-OC_2H_5	(239)
2-Br, 6-NH_2	(239)
	(84)

B. Attempted syntheses of unsymmetrical biaryls

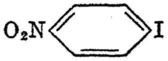
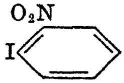
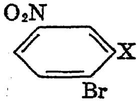
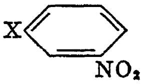
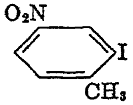
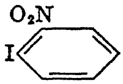
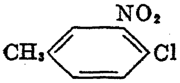
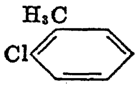
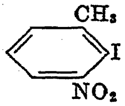
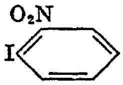
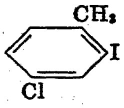
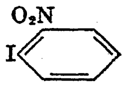
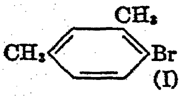
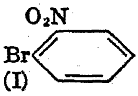
REACTANTS		REFERENCE
		(60a)
		(36b)
		(119)
		(128)
		(128)
		(128)
		(128)

TABLE 3—*Continued*

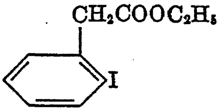
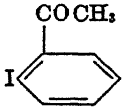
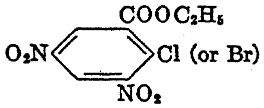
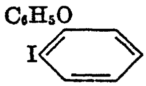
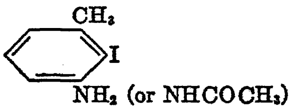
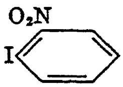
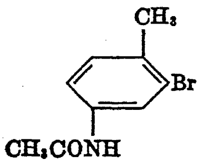
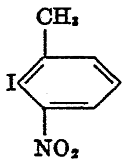
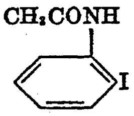
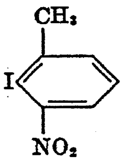
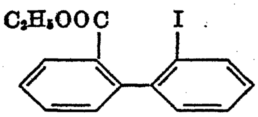
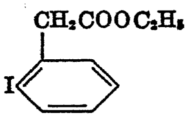
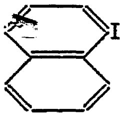
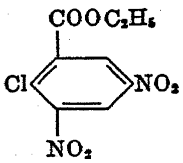
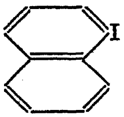
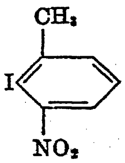
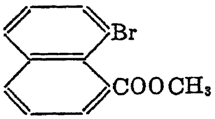
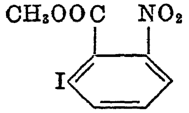
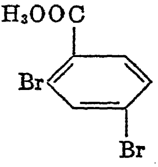
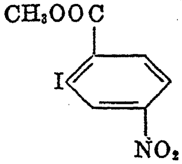
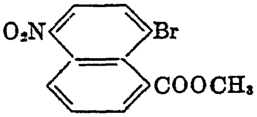
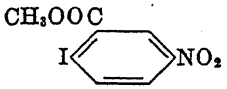
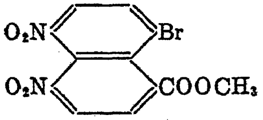
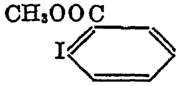
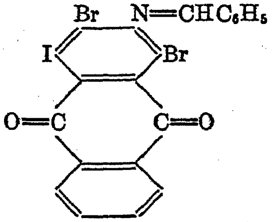
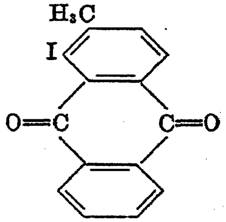
REACTANTS		REFERENCE
		(153)
		(111)
		(15)
		(15)
		(15)
		(184a)
		(111)
		(15)

TABLE 3—*Concluded*

REACTANTS		REFERENCE
		(155a)
		(155a)
		(155a)
		(155a)
		(155a)
		(165)

VI. REFERENCES

(1) Adams, R., and Cairns, T. L.: J. Am. Chem. Soc. **61**, 2179–81 (1939).
(2) Adams, R., and Finger, G. C.: J. Am. Chem. Soc. **61**, 2828–30 (1939).
(3) Adams, R., Geissman, T. A., Baker, R. B., and Teeter, H. M.: J. Am. Chem. Soc. **63**, 528–34 (1941).
(4) Adams, R., and Hale, J. B.: J. Am. Chem. Soc. **61**, 2825–8 (1939).
(5) Adams, R., and Snyder, H. R.: J. Am. Chem. Soc. **60**, 1411–15 (1938).
(6) Adams, R., and Teeter, H. M.: J. Am. Chem. Soc. **62**, 2188–90 (1940).
(6a) Arnold, R. T., and Coyner, E. C.: J. Am. Chem. Soc. **66**, 1542–5 (1944).
(7) Bachmann, W. E.: J. Am. Chem. Soc. **54**, 1969–74 (1932).
(8) Bachmann, W. E.: J. Am. Chem. Soc. **56**, 1363–7 (1934).
(9) Bachmann, W. E., and Chu, E. J.-H.: J. Am. Chem. Soc. **57**, 1095–8 (1935).

(10) Bachmann, W. E., and Clarke, H. T.: J. Am. Chem. Soc. **49**, 2089–98 (1927).
(10a) Baddar, F. G.: J. Chem. Soc. **1941**, 310–12.
(10b) Baddar, F. G., and Gindy, M.: J. Chem. Soc. **1944**, 450–2.
(10c) Baddar, F. G., and Warren, F. L.: J. Chem. Soc. **1939**, 944–8.
(10d) Baddar, F. G., and Warren, F. L.: J. Chem. Soc. **1938**, 401–4.
(11) Badische Anilin- und Soda-Fabrik: German patent 175,067 (1905); Chem. Abstracts **1**, 1085 (1907).
(11a) Badische Anilin- und Soda-Fabrik: German patent 180,157 (1907); Chem. Zentr. **78**, I, 680 (1907).
(11b) Badische Anilin- und Soda-Fabrik: German patent 184,495 (1907); Chem. Zentr. **78**, II, 434 (1907).
(12) Badische Anilin- und Soda-Fabrik: German patent 189,157 (1905); Chem. Abstracts **1**, 1653 (1907).
(12a) Badische Anilin- und Soda-Fabrik: German patent 241,472 (1911); Chem. Zentr. **1912**, I, 174.
(13) Barber, H. J., and Smiles, S.: J. Chem. Soc. **1928**, 1141–9.
(14) Becker, B. C., and Adams, R.: J. Am. Chem. Soc. **54**, 2973–82 (1932).
(15) Bell, F.: J. Chem. Soc. **1934**, 835–8.
(16) Bell, F., and Kenyon, J.: J. Chem. Soc. **1926**, 2705–13.
(17) Benesch, E.: Monatsh. **32**, 447–56 (1911).
(18) Bock, L. H., Moyer, W. W., and Adams, R.: J. Am. Chem. Soc. **52**, 2054–60 (1930).
(19) Boedtker, E.: Bull. soc. chim. [4] **45**, 645–50 (1929).
(20) Borsche, W., and Rantscheff, D.: Ann. **379**, 152–82 (1911).
(21) Bowden, S. T.: J. Chem. Soc. **1931**, 1111–14.
(21a) Bradsher, C. K., and Amore, S. T.: J. Am. Chem. Soc. **66**, 1283–4 (1944).
(22) Brady, O. L., and McHugh, G. P.: J. Chem. Soc. **1923**, 2047–53.
(23) Brand, K., and Groebe, W.: J. prakt. Chem. **108**, 1–18 (1924).
(24) Brand, K., and Stallmann, O.: J. prakt. Chem. **107**, 358–82 (1924).
(25) Brass, K., and Patzelt, R.: Ber. **70**, 1349–53 (1937).
(26) Britton, E. C., and Livak, J. E.: U. S. patent 2,260,739 (1941); Chem. Abstracts **36**, 911 (1942).
(27) Britton, E. C., and Livak, J. E.: U. S. patent 2,285,563 (1942); Chem. Abstracts **36**, 6814 (1942).
(28) Burstall, F. H.: J. Chem. Soc. **1938**, 1662–72.
(29) Burton, H., and Kenner, J.: J. Chem. Soc. **1922**, 489–96.
(30) Burton, H., and Kenner, J.: J. Chem. Soc. **1923**, 1043–5.
(31) Burton, H., Hammond, F., and Kenner, J.: J. Chem. Soc. **1926**, 1802–4.
(32) Cahn, R. S.: J. Chem. Soc. **1933**, 1400–5.
(33) Cain, J. C.: J. Chem. Soc. **1904**, 7–11.
(33a) Carlin, R. B.: J. Am. Chem. Soc. **67**, 928–33 (1945).
(34) Case, F. H.: J. Am. Chem. Soc. **60**, 424–7 (1938).
(35) Case, F. H.: J. Am. Chem. Soc. **61**, 3487–90 (1939).
(36) Case, F. H.: J. Am. Chem. Soc. **64**, 1848–52 (1942).
(36a) Case, F. H.: J. Am. Chem. Soc. **64**, 2225 (1942).
(36b) Case, F. H.: J. Am. Chem. Soc. **65**, 2137 (1943).
(37) Case, F. H., and Koft, E.: J. Am. Chem. Soc. **63**, 508–10 (1941).
(37a) Case, F. H., and Schock, R. U.: J. Am. Chem. Soc. **65**, 2086–8 (1943).
(38) Chien, S. L., and Adams, R.: J. Am. Chem. Soc. **56**, 1787–92 (1934).
(39) Christie, G. H., James, C. W., and Kenner, J.: J. Chem. Soc. **1923**, 1948–51.
(39a) Christie, G. H., and Kenner, J.: J. Chem. Soc. **1922**, 614–20.
(40) Chudozilov, L. K.: Chem. Listy **19**, 187–90 (1925); Chem. Abstracts **19**, 3268 (1925).
(41) Clemo, G. R., Cockburn, J. G., and Spence, R.: J. Chem. Soc. **1931**, 1265–73.
(42) Conolly, E. J. V.: J. Chem. Soc. **1925**, 2083–5.
(43) Cornforth, J. W., and Robinson, R.: J. Chem. Soc. **1942**, 684–9.

(44) Crossley, A. W., and Hampshire, C. H.: J. Chem. Soc. **1911**, 721–6.

(45) Cumming, W. M., and Howie, G.: J. Chem. Soc. **1931**, 3176–81.

(46) Cumming, W. M., and Muir, G. D.: J. Roy. Tech. Coll. (Glasgow) **4**, 61–71 (1937); Chem. Abstracts **31**, 4310 (1937).

(47) de Diesbach, H., and Janzen, T.: Helv. Chim. Acta **11**, 724–30 (1928).

(48) Dennett, H. G., and Turner, E. E.: J. Chem. Soc. **1926**, 476–81.

(49) Dobbie, J. J., Fox, J. J., and Gauge, A. J. H.: J. Chem. Soc. **1913**, 36–41.

(50) Dutt, S.: J. Chem. Soc. **1926**, 1171–84.

(51) Eckert, A., and Endler, G.: J. prakt. Chem. **104**, 91–101 (1922).

(52) Eckert, A., and Hampel, J.: Ber. **60**, 1693–1703 (1927).

(53) Eckert, A., and Tomaschek, R.: Monatsh. **39**, 839–64 (1918).

(54) Erdtman, H.: Ann. **505**, 195–202 (1933).

(55) Erdtman, H.: Ann. **513**, 240–50 (1934).

(56) Erdtmann, H. G. H.: Proc. Roy. Soc. (London) **A143**, 177–91 (1933).

(57) Erlenmeyer, H., and Schmid, E. H.: Helv. Chim. Acta **22**, 698–700 (1939).

(58) Faber, A. C., and Nauta, W. T.: Rec. trav. chim. **62**, 469–84 (1943); Chem. Abstracts **38**, 4576–81 (1944).

(59) Fabinyi, R., and Szecki, T.: Ber. **43**, 2676–84 (1910).

(60) Finger, H., and Schott, W.: J. prakt. Chem. **115**, 281–91 (1927).

(60a) Finzi, C., and Bellavita, V.: Gazz. chim. ital. **64**, 335–45 (1934); Chem. Abstracts **28**, 5429 (1934).

(61) Fuson, R. C., and Cleveland, E. A.: Org. Syntheses **20**, 45–7 (1940).

(62) Gerngross, O., and Dunkel, M.: Ber. **57**, 739–46 (1924).

(63) Gilman, H., and Straley, J. M.: Rec. trav. chim. **55**, 821–34 (1936).

(63a) Gilman, H., and Thirtle, J. R.: J. Am. Chem. Soc. **66**, 858–9 (1944).

(64) Goldschmidt, S., and Schön, W.: Ber. **59**, 948–55 (1926).

(65) Grieve, W. S. M., and Hey, D. H.: J. Chem. Soc. **1932**, 1888–94.

(66) Gull, H. C., and Turner, E. E.: J. Chem. Soc. **1929**, 491–500.

(67) Hanford, W. E., and Adams, R.: J. Am. Chem. Soc. **57**, 1592–5 (1935).

(68) Hannon, J., and Kenner, J.: J. Chem. Soc. **1934**, 138.

(69) Hardacre, R. W., and Perkin, A. G.: J. Chem. Soc. **1929**, 180–93.

(70) Harley-Mason, J., and Mann, F. G.: J. Chem. Soc. **1940**, 1379–85.

(71) Hata, K., Tatematsu, K., and Kubota, B.: Bull. Chem. Soc. Japan **10**, 425–32 (1935); Chem. Abstracts **30**, 1056 (1936).

(72) Hodgson, H. H., and Elliott, R. L.: J. Chem. Soc. **1937**, 123–5.

(73) Hodgson, H. H., and Crook, J. H.: J. Chem. Soc. **1937**, 571–3.

(73a) Huebner, C. F., and Link, K. P.: J. Am. Chem. Soc. **67**, 99–102 (1945).

(74) Hughes, G. K., Lions, F., Maunsell, J. J., and Wright, L. E. A.: J. Proc. Roy. Soc. N. S. Wales **71**, 428–34 (1938); Chem. Abstracts **33**, 613 (19[illegible]

(75) Ioffe, I. S., and Grachev, I. U.: J. Gen. Chem. (U.S.S.R.) **3**, 463–9 (1933); Chem. Abstracts **28**, 1692 (1934).

(76) I. G. Farbenindustrie A.-G.: German patent 551,447 (1930); Chem. Abstracts **26**, 4480 (1932).

(77) I. G. Farbenindustrie A.-G.: German patent 558,474 (1930); Chem. Abstracts **27**, 309 (1933).

(78) I. G. Farbenindustrie A.-G.: German patent 564,788 (1931); Chem. Abstracts **27**, 1000 (1933).

(79) I. G. Farbenindustrie A.-G.: German patent 567,755 (1931); Chem. Abstracts **27**, 2693 (1933).

(80) I. G. Farbenindustrie A.-G.: French patent 729,575 (1931); Chem. Abstracts **27**, 309 (1933).

(81) I. G. Farbenindustrie A.-G.: British patent 389,568 (1933); Chem. Abstracts **27**, 4935 (1933).

(82) Ingersoll, A. W., and Little, J. R.: J. Am. Chem. Soc. **56**, 2123–6 (1934).
(83) Isler, M. H.: U. S. patent 1,004,433 (1911); Chem. Abstracts **6**, 300 (1912).
(84) John, H.: Ber. **59**, 2709–12 (1926).
(85) Kalb, L.: Ber. **47**, 1724–30 (1914).
(86) Kalb, L.: German patent 280,787 (1914); Chem. Zentr. **1915**, I, 75–6.
(87) Kenner, J.: Proc. Chem. Soc. **28**, 277 (1912); Chem. Abstracts **7**, 2932 (1913).
(88) Kenner, J., and Mathews, A. M.: J. Chem. Soc. **1914**, 2471–82.
(89) Kenner, J., and Stubbings, W. V.: J. Chem. Soc. **1921**, 593–602.
(90) Kenner, J., and Turner, E. G.: J. Chem. Soc. **1911**, 2101–14.
(91) Kenner, J., and Turner, H. A.: J. Chem. Soc. **1928**, 2340–3.
(92) Kenner, J., and Witham, E.: J. Chem. Soc. **1913**, 232–8.
(93) Kleiderer, E. C., and Adams, R.: J. Am. Chem. Soc. **53**, 1575–80 (1931).
(94) Kleiderer, E. C., and Adams, R.: J. Am. Chem. Soc. **55**, 716–19 (1933).
(95) Kleiderer, E. C., and Adams, R.: J. Am. Chem. Soc. **55**, 4219–25 (1933).
(96) Kondo, H., and Ikeda, T.: Ber. **73**, 867–74 (1940).
(97) Kopetschni, E.: German patents 360,419 and 362,984; Chem. Abstracts **18**, 991 (1924).
(98) Kopetschni, E.: German patent 363,930; Chem. Abstracts **18**, 991 (1924).
(99) Korolev, A., and Bilik, I.: Compt. rend. acad. sci. U.R.S.S. **29**, 586–8 (1940); Chem. Abstracts **35**, 6795 (1941).
(100) Korczynski, A., Karlowska, G., and Kierzek, L.: Bull. soc. chim. [4] **41**, 65–74 (1927).
(101) Kuhn, R., and Albrecht, O.: Ann. **455**, 272–99 (1927).
(102) Kuhn, R., and Albrecht, O.: Ann. **458**, 221–9 (1927).
(103) Kuhn, R., and Albrecht, O.: Ann. **464**, 91–100 (1928).
(104) Kuhn, R., and Albrecht, O.: Ann. **465**, 282–7 (1928).
(105) Le Fevre, R. J. W.: J. Chem. Soc. **1929**, 733–8.
(106) Le Fevre, R. J. W., and Vine, H.: J. Chem. Soc. **1938**, 967–72.
(107) Legler, H.: German patent 536,081 (1930); Chem. Abstracts **26**, 1001 (1932).
(108) Lehmstedt, K., and Hundertmark, H.: Ber. **62**, 1065–7 (1929).
(109) Lesslie, M. S., and Turner, E. E.: J. Chem. Soc. **1930**, 1758–65.
(110) Lesslie, M. S., and Turner, E. E.: J. Chem. Soc. **1931**, 1188–92.
(111) Lesslie, M. S., and Turner, E. E.: J. Chem. Soc. **1932**, 281–5.
(112) Lesslie, M. S., and Turner, E. E.: J. Chem. Soc. **1932**, 2021–6.
(113) Li, C. C., and Adams, R.: J. Am. Chem. Soc. **57**, 1565–9 (1935).
(114) Liebermann, C.: Ber. **44**, 1453–5 (1911).
(115) Liebermann, C., and Kardos, M.: Ber. **46**, 198–212 (1913).
(116) Longo, B.: Atti accad. sci. Torino, Classe sci. fis. mat. nat. **73**, 434–6 (1938); Chem. Abstracts **33**, 6286 (1939).
(117) Longo, B.: Atti accad. sci. Torino, Classe sci. fis. mat. nat. **73**, 437–9 (1938); Chem. Abstracts **33**, 6286 (1939).
(118) Longo, B.: Atti accad. sci. Torino, Classe sci. fis. mat. nat. **73**, 440–2 (1938); Chem. Abstracts **33**, 6286 (1939).
(119) Longo, B.: Atti cong. naz. chim. pura applicata, 5th Congr., Rome, **1935**, Pt. I, 386–8 (published 1936); Chem. Abstracts **31**, 3902 (1937).
(120) Lothrop, W. C.: J. Am. Chem. Soc. **63**, 1187–91 (1941).
(121) Lothrop, W. C.: J. Am. Chem. Soc. **64**, 1698–1700 (1942).
(122) Lüttringhaus, A., and Neresheimer, H.: Ann. **473**, 259–89 (1929).
(123) Marler, E. E. J., and Turner, E. E.: J. Chem. Soc. **1932**, 2391–4.
(124) Martin, R. H.: J. Chem. Soc. **1941**, 679–85.
(125) Mascarelli, L., and Gatti, D.: Atti cong. naz. chim. pura applicata, 4th Congr. **1932**, 503–5 (published 1933); Chem. Abstracts **29**, 4351 (1935).
(126) Mascarelli, L., Gatti, D., and Longo, B.: Gazz. chim. ital. **63**, 661–5 (1933); Chem. Abstracts **28**, 1686 (1934).

(127) Mascarelli, L., Gatti, D., and Pirona, M.: Gazz. chim. ital. **61**, 782–97 (1931); Chem. Abstracts **26**, 1595–7 (1932).

(128) Mascarelli, L., and Longo, B.: Gazz. chim. ital. **67**, 812–16 (1937); Chem. Abstracts **32**, 4564–5 (1938).

(129) Mascarelli, L., and Longo, B.: Atti accad. Lincei, Classe sci. fis. mat. nat. **26**, 292–7 (1937); Gazz. chim. ital. **68**, 121–9 (1938); Chem. Abstracts **32**, 6235–6 (1938).

(130) Mascarelli, L., and Longo, B.: Gazz. chim. ital. **71**, 289–93 (1941); Chem. Abstracts **37**, 1415 (1943).

(131) Mascarelli, L., and Longo, B.: Gazz. chim. ital. **71**, 389–406 (1941); Chem. Abstracts **37**, 1415 (1943).

(132) Mascarelli, L., Longo, B., and Ravera, A.: Gazz. chim. ital. **68**, 33–48 (1938); Chem. Abstracts **32**, 4565 (1938).

(133) Mayer, F.: Ber. **44**, 2298–2305 (1911).

(134) Mayer, F.: Ber. **45**, 1105–13 (1912).

(135) Mayer, F.: Ber. **47**, 406–11 (1914).

(136) Mayer, F., and Freitag, K.: Ber. **54**, 347–57 (1921).

(137) McAlister, F. B., and Kenner, J.: J. Chem. Soc. **1928**, 1913–16.

(138) Meisenheimer, J., and Beisswenger, O.: Ber. **65**, 32–42 (1932).

(139) Meisenheimer, J., and Höring, M.: Ber. **60**, 1425–33 (1927).

(140) Meyer, R., Meyer, W., and Taeger, K.: Ber. **53**, 2034–52 (1920).

(141) Miller, H. F., and Bachmann, G. B.: J. Am. Chem. Soc. **57**, 2443–6 (1935).

(142) Minaev, V.: Chem.-Ztg. **36**, 199 (1912); Chem. Abstracts **7**, 1481 (1913).

(143) Minaev, V., and Ripper, K.: Monatsh. **42**, 73–81 (1921); also J. Russ. Phys.-Chem. Soc. **54**, 829–32 (1924); Chem. Abstracts **19**, 3269 (1925).

(144) Moser, W., and Fioroni, W.: U. S. patent 2,002,247 (1935); Chem. Abstracts **29**, 4382 (1935).

(145) Moyer, W. W., and Adams, R.: J. Am. Chem. Soc. **51**, 630–8 (1929).

(146) Müller, E., and Tietz, E.: Ber. **74**, 807–24 (1941); Chem. Abstracts **35**, 7952–5 (1941).

(147) Patterson, W. I., and Adams, R.: J. Am. Chem. Soc. **57**, 762–4 (1935).

(148) Perkin, A. G., and Haddock, N. H.: J. Chem. Soc. **1933**, 1512–20.

(148a) Posternak, T., Ruelius, H. W., and Tcherniak, J.: Helv. Chim. Acta **26**, 2031–44 (1943); Chem. Abstracts **38**, 1218 (1944).

(149) Pufahl, F.: Ber. **62**, 2817–24 (1929).

(150) Pummerer, R., and Bittner, K.: Ber. **57**, 84–8 (1924).

(151) Pummerer, R., Puttfarçken, H., and Schopflocher, P.: Ber. **58**, 1808–20 (1925).

(152) Pummerer, R., and Seligsberger, L.: Ber. **64**, 2477–86 (1931).

(153) Rapson, W. S., and Shuttleworth, R. G.: J. Chem. Soc. **1941**, 487–90.

(153a) Rapson, W. S., and Shuttleworth, R. G.: Nature **147**, 675 (1941).

(154) Rindl, M.: J. Chem. Soc. **1913**, 1911–17.

(155) Ruggli, P., and Merz, E.: Helv. Chim. Acta **12**, 71–99 (1929).

(155a) Rule, H. G., and Smith, F. R.: J. Chem. Soc. **1937**, 1096–1103.

(155b) Rule, H. G., Pursell, W., and Barnett, A. J. G.: J. Chem. Soc. **1935**, 571–2.

(156) Sadler, A. M., and Powell, G.: J. Am. Chem. Soc. **56**, 2650–3 (1934).

(157) Sako, S.: Mem. Coll. Eng. Kyushu Imp. Univ. **6**, 263–306 (1932); Chem. Abstracts **26**, 3246–8 (1932).

(158) Sako, S.: Bull. Chem. Soc. Japan **10**, 585–92 (1935); Chem. Abstracts **30**, 2560 (1936).

(159) Sako, S.: Bull. Chem. Soc. Japan **10**, 593–4 (1935); Chem. Abstracts **30**, 2560 (1936).

(160) Sako, S.: Bull. Chem. Soc. Japan **11**, 144–57 (1936); Chem. Abstracts **30**, 5984 (1936).

(161) Schiemann, G., and Roselius, W.: Ber. **65**, 737–45 (1932).

(162) Scholl, R.: Ber. **40**, 1691–1702 (1907).

(163) Scholl, R.: Ber. **43**, 346–56 (1910).

(164) Scholl, R.: Ber. **52**, 1829–36 (1919).

(165) Scholl, R., and Dischendorfer, O.: Ber. **51**, 441–52 (1918).

(166) Scholl, R., and Dischendorfer, O.: Ber. **51**, 452–3 (1918).

(167) Scholl, R., Liese, K., Michelson, K., and Grunewald, E.: Ber. **43**, 512–18 (1910).

(168) Scholl, R., and Mansfeld, J.: Ber. **43**, 1734–46 (1910).

(169) Scholl, R., and Meyer, H. K.: Ber. **68**, 1307–9 (1935).

(170) Scholl, R., Müller, E. J., and Böttger, O.: Ber. **68**, 45–9 (1935).

(171) Scholl, R., and Neovius, W.: Ber. **44**, 1075–90 (1911).

(172) Scholl, R., and Neuberger, W.: Monatsh. **33**, 507–33 (1912).

(173) Scholl, R., Potschiwauscheg, J., and Lenko, J.: Monatsh. **32**, 687–710 (1911).

(173a) Scholl, R., Seer, C., and Weitzenböck, R.: Ber. **43**, 2202–9 (1910).

(174) Scholl, R., Seer, C., Weitzenböck, R., and Ertl, A.: Monatsh. **42**, 405–9 (1921).

(175) Scholl, R., with Tritsch, W.: Monatsh. **39**, 231–6 (1918).

(176) Schlubach, H. H., and Mergenthaler, F.: Ber. **58**, 2732–6 (1925).

(177) Schoepfle, C. S.: J. Am. Chem. Soc. **45**, 1566–71 (1923).

(178) Schreiner, E.: J. prakt. Chem. **81**, 422–4 (1910).

(179) Schwenk, E., and Waldmann, H.: J. prakt. Chem. **130**, 79–80 (1931).

(180) Searle, N. E., and Adams, R.: J. Am. Chem. Soc. **55**, 1649–54 (1933).

(181) Seer, C., and Karl, E.: Monatsh. **34**, 631–48 (1913).

(182) Seer, C., and Scholl, R.: Ann. **398**, 82–96 (1913).

(183) Sen, R. N., and Dutt, S.: J. Indian Chem. Soc. **8**, 223–9 (1931); Chem. Abstracts **26**, 127–8 (1932).

(183a) Shaw, F. R., and Turner, E. E.: J. Chem. Soc. **1932**, 285–97.

(184) Shaw, F. R., and Turner, E. E.: J. Chem. Soc. **1932**, 509–13.

(184a) Shuttleworth, R. G., Rapson, W. S., and Stewart, E. T.: J. Chem. Soc. **1944**, 71–3.

(185) Simada, T., and Hata, K.: Sci. Papers Inst. Phys. Chem. Research (Tokyo) **35**, 365–71 (1939); Chem. Abstracts **33**, 4594–5 (1939).

(186) Sircar, A. C., and Majumdar, J. N.: J. Indian Chem. Soc. **5**, 417–18 (1928); Chem. Abstracts **23**, 129 (1929).

(187) Smith, W., Thomas, J., and Scottish Dyes: British patent 336,982 (1929); Chem. Abstracts **25**, 2004 (1931).

(188) Smith, W., and Thomas, J.: U. S. patent 1,834,876 (1931); Chem. Abstracts **26**, 1452 (1932).

(189) Smith, W., and Thomas, J.: U. S. patent 1,890,098 (1932); Chem. Abstracts **27**, 1765 (1933).

(190) Société pour l'industrie chimique à Bâle: French patent 786,363 (1935); Chem. Abstracts **30**, 1072 (1936).

(191) Société pour l'industrie chimique à Bâle: British patent 450,700 (1936); Chem. Abstracts **31**, 268–9 (1937).

(192) Société pour l'industrie chimique à Bâle: British patent 499,836 (1939); Chem. Abstracts **33**, 5202 (1939).

(193) Späth, E., and Gibian, K.: Monatsh. **55**, 342–51 (1930).

(194) Sprengstoff: British patent 18,333 (1914); Chem. Abstracts **10**, 392 (1916).

(195) Stanley, W. M., and Adams, R.: J. Am. Chem. Soc. **53**, 2364–8 (1931).

(196) Stanley, W. M., McMahon, E., and Adams, R.: J. Am. Chem. Soc. **55**, 706–16 (1933).

(197) Stearns, H. A., and Adams, R.: J. Am. Chem. Soc. **52**, 2070–5 (1930).

(197a) Steinkopf, W.: Ann. **543**, 128–32 (1940).

(198) Steinkopf, W., Barlag, T., and von Petersdorff, H.-J.: Ann. **540**, 7–14 (1939).

(199) Steinkopf, W., and Hanske, W.: Ann. **541**, 238–60 (1939).

(200) Steinkopf, W., and Jaeger, P.: J. prakt. Chem. **128**, 63–88 (1930).

(201) Steinkopf, W., Leitsmann, R., and Hofmann, K. H.: Ann. **546**, 180–99 (1941).

(202) Steinkopf, W., Leitsmann, R., Müller, A. H., and Wilhelm, H.: Ann. **541**, 260–82 (1939).

(203) Steinkopf, W., Merckoll, A., and Strauch, H.: Ann. **545**, 45–51 (1940).

(204) Steinkopf, W., and von Petersdorff, H.-J.: Ann. **543**, 119–28 (1940).

(205) Steinkopf, W., von Petersdorff, H.-J., and Gording, R.: Ann. **527**, 272–8 (1937).

(206) STOUGHTON, R. W., AND ADAMS, R.: J. Am. Chem. Soc. **52**, 5263–7 (1930).
(207) STOUGHTON, R. W., AND ADAMS, R.: J. Am. Chem. Soc. **54**, 4426–34 (1932).
(208) SUGII, Y.: J. Pharm. Soc. Japan **50**, 183–217 (1930); Chem. Abstracts **24**, 3505 (1930) (evidently erroneous; for a better abstract see Chem. Zentr. **1930**, II, 253–4).
(209) SUGII, Y., AND SHINDO, H.: J. Pharm. Soc. Japan **54**, 829–44 (1934); Chem. Abstracts **29**, 790–1 (1935).
(210) TANAKA, M.: Chem. News **131**, 49–50 (1925); Chem. Abstracts **19**, 2948 (1925).
(210a) TARBELL, D. S., FRANK, H. R., AND FANTA, P. E.: J. Am. Chem. Soc. **68**, in press (1946).
(211) TSCHITSCHIBABIN, A. E.: Ber. **40**, 1810–19 (1907).
(212) TSCHITSCHIBABIN, A. E.: J. Russ. Phys.-Chem. Soc. **39**, 925–36 (1907); Chem. Abstracts **2**, 405–6 (1908).
(213) TEETER, H. M.: Trans. Illinois State Acad. Sci. **33**, No. 2, 120–1 (1940); Chem. Abstracts **35**, 2495 (1941).
(214) UEO (or UYEO), S.: Ber. **73**, 661–9 (1940).
(215) ULLMANN, F.: Ber. **29**, 1878–81 (1896).
(216) ULLMANN, F.: Ann. **332**, 38–81 (1904).
(217) ULLMANN, F.: Ann. **366**, 79–118 (1909).
(218) ULLMANN, F.: British patent 2,949 (1912); Chem. Abstracts **7**, 2477–8 (1913).
(219) ULLMANN, F., AND BIELECKI, J.: Ber. **34**, 2174–85 (1901).
(220) ULLMANN, F., AND EISER, O.: Ber. **49**, 2154–69 (1916).
(221) ULLMANN, F., AND FRENTZEL, L.: Ber. **38**, 725–9 (1905).
(222) ULLMANN, F., AND VON GLENK, O.: Ber. **49**, 2487–2514 (1916).
(223) ULLMANN, F., AND JUNGHAUS, W.: Ann. **399**, 330–45 (1913).
(224) ULLMANN, F., AND MINAJEFF, W.: Ber. **45**, 687–90 (1912).
(225) VAN ALPHEN, J.: Rec. trav. chim. **50**, 1111–6 (1931).
(226) VAN ALPHEN, J.: Rec. trav. chim. **51**, 361–8 (1932).
(227) VAN ALPHEN, J.: Rec. trav. chim. **51**, 453–9 (1932).
(228) VAN ARENDONK, A. M., BECKER, B. C., AND ADAMS, R.: J. Am. Chem. Soc. **55**, 4230–4 (1933).
(229) VAN ARENDONK, A. M., CUPERY, M. E., AND ADAMS, R.: J. Am. Chem. Soc. **55**, 4225–30 (1933).
(229a) VAN DUIN, C. F.: Rec. trav. chim. **39**, 685–8 (1920).
(230) VAN ROOSMALEN, F. L. W.: Rec. trav. chim. **53**, 359–79 (1934).
(231) VESELY, V.: Ber. **38**, 136–9 (1905).
(232) WALDMANN, H., AND POLAK, G.: J. prakt. Chem. **150**, 113–20 (1938).
(233) WARD, H. P., AND WARING, M. G.: J. Am. Chem. Soc. **54**, 1697 (1932).
(234) WEITZENBÖCK, R.: Monatsh. **34**, 193–223 (1913).
(235) WEITZENBÖCK, R.: Monatsh. **39**, 307–13 (1918).
(236) WHITE, J.: Thesis, University of Illinois, 1931; Chem. Abstracts **27**, 1358 (1933).
(237) WHITE, J., AND ADAMS, R.: J. Am. Chem. Soc. **54**, 2104–8 (1932).
(238) WIBAUT, J. P., AND OVERHOFF, J.: Rec. trav. chim. **47**, 761–3 (1928).
(239) WILLINK, H. D. T., AND WIBAUT, J. P.: Rec. trav. chim. **54**, 275–83 (1935).
(239a) WILLIS, H. B.: Iowa State Coll. J. Sci. **18**, 98–101 (1943); Chem. Abstracts **38**, 739–40 (1944).
(240) WITTIG, G., AND STICHNOTH, O.: Ber. **68**, 928–35 (1935).
(241) YUAN, H. C., AND ADAMS, R.: J. Am. Chem. Soc. **54**, 2966–73 (1932).
(242) YUAN, H. C., AND ADAMS, R.: J. Am. Chem. Soc. **54**, 4434–43 (1932).
(243) YUAN, H. C., AND HSU, T.-T.: J. Chinese Chem. Soc. **3**, 206–12 (1935); Chem. Abstracts **29**, 7313 (1935).
(244) YUAN, H. C., AND TSAO, M.: J. Chinese Chem. Soc. **3**, 358–64 (1935); Chem. Abstracts **30**, 2187 (1936); correction, Chem. Abstracts **30**, 7095 (1936).
(245) ZAHN, K., AND OCHWAT, P.: U. S. patent 1,675,014 (1928); Chem. Abstracts **22**, 3050 (1928).
(246) ZINKE, A., AND ZIEGLER, E.: Ber. **74**, 115–18 (1941).

OXIDATION OF LUBRICATING OILS

H. H. ZUIDEMA

Research Laboratories, Shell Oil Company, Inc., Wood River, Illinois

Received October 15, 1945

CONTENTS

I. Introduction 197
II. Oxidation products 198
  A. Pure hydrocarbons 198
  B. Mixtures and petroleum fractions 207
III. Oxidation rates 212
  A. Pure hydrocarbons 212
  B. Mixtures and petroleum fractions 216
  C. Effect of catalysts and inhibitors 220
IV. Conclusions 223
V. References 225

Mechanisms are proposed for the oxidation of three types of hydrocarbons—paraffin, naphthene, and aromatic—under conditions comparable to those encountered by lubricating oils in service. The initial oxidation product is postulated to be a hydroperoxide, which decomposes to form acids, ketones, aldehydes, and other intermediate oxidation products. These products may in turn undergo further oxidation or condensation. Condensation products are particularly prevalent in the case of aromatics.

The effect of hydrocarbon structure upon rate of oxidation is discussed. Paraffins and naphthenes oxidize at an intermediate rate. The introduction of olefinic unsaturation or a partially hydrogenated aromatic ring into the molecule increases its rate of oxidation, as does the addition of a benzene ring to the end of a long paraffin molecule. The presence of a naphthalene nucleus has the opposite effect of stabilizing the molecule.

Interaction effects play an important rôle in determining the rate of oxidation of a complex mixture of hydrocarbons, e.g., a lubricating oil. The rate is not, in general, the mean of the rates of the individual components, but is usually quite different. The component which is the most stable when tested alone may be preferentially oxidized in a solution of several components.

The catalytic effect of compounds of copper, lead, and iron is discussed. These compounds, which may be introduced in low concentration into a lubricating oil through contact with the metals, often exert a large accelerating effect upon the over-all rate of oxidation.

I. INTRODUCTION

Modern engines are exacting in their lubrication requirements. An extremely flat viscosity–temperature curve may be needed, or anti-rust characteristics, or good fluid characteristics at low temperatures, or any one or more of numerous other properties. While oils for various applications must meet widely different specifications, they have one requirement in common—they should undergo a minimum of change during use. Changes in properties during operation are due partly to extraneous contamination, but largely to chemical changes in the oil molecules themselves. These chemical reactions are principally those involving oxidation.

Oxidation of a lubricant in some cases benefits certain properties. For example, King (28) found, in a test employing a journal bearing at a pressure of 1000 lb. per square inch of projected area, that preoxidation of a mineral oil reduced its coefficient of friction and increased its "critical seizure temperature" by 145°C. However, the same effect could probably have been realized by the addition of a small amount of polar compound, without the attendant disadvantages of the oxidation treatment. Another example is cited by Baker (1), who reports that the addition of 10 per cent of used turbine oil to a fresh charge imparts rust-preventive properties not present in the fresh oil. This method of rust prevention is not without penalty, however, since other properties of the oil, notably service life and the ability to separate entrained moisture, are drastically damaged. It has accordingly been largely supplanted by the incorporation of a trace of highly effective compound. These additive-type oils are much more effective as rust preventives than the previous blends of new and oxidized oil, and the additive has very little effect upon other properties.

Oxidation of a lubricating oil leads to such difficulties as bearing corrosion, ring sticking, lacquer and sludge formation, and excessive viscosity. It is sometimes possible to overcome the harmful effect of a given class of oxidation products. An example of this approach to the problem is the addition of detergents, which peptize colloidal oxidation products and prevent their deposition as lacquer. Another example is the use of certain sulfur-containing additives which, in addition to performing other functions, passivate bearing surfaces and thus prevent corrosive acids which may be present in the oil from attacking the bearings. While methods such as these are commonly used, the over-all control of oxidation is nevertheless essential, not so much to eliminate the need for specific additives other than antioxidants, but to reduce the burden imposed upon them.

The purpose of the present paper is to review the literature on the subject of oxidation of hydrocarbons and lubricating oils, with the aim of gaining a clearer insight into the mechanisms involved.

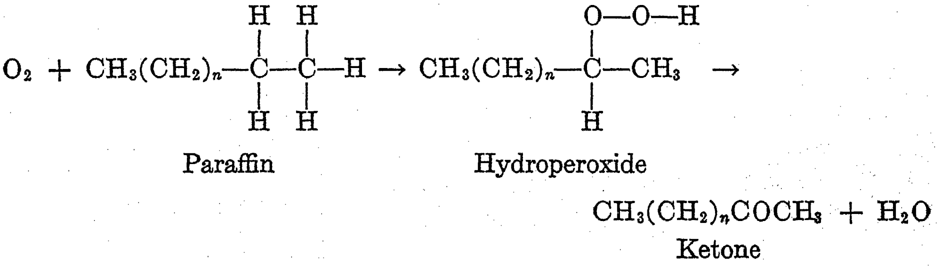
II. OXIDATION PRODUCTS

A. Pure hydrocarbons

Lubricating oils are composed of such a complex mixture of hydrocarbons that it is extremely difficult to identify specific compounds in their oxidation products, except for such degradation products as water, carbon dioxide, and some of the lower carboxylic acids. However, since lubricating oils are composed primarily of hydrocarbons, including three main groups—namely, paraffin, naphthene, and aromatic—it is of interest to examine the literature on the oxidation of pure hydrocarbons of these three types under conditions comparable to those encountered by oils in actual use.

Chavanne and coworkers (4, 5, 17) have studied the oxidation of a few paraffins and naphthenes. They oxidized *n*-decane, *n*-nonane, and *n*-octane with oxygen at atmospheric pressure and a temperature of 120°C. The gaseous oxidation products, which account for 10 per cent or so of the total, were similar in all three cases, and included 30–40 per cent of carbon dioxide, 1–3 per cent of carbon monoxide, 5–7 per cent of hydrogen, and 1–2 per cent of saturated hydrocarbons. The liquid products contained water, succinic acid, and formaldehyde. The three hydrocarbons produced preponderant amounts of methyl octyl, methyl heptyl, and methyl hexyl ketones, respectively, as well as a series of carboxylic acids ranging from formic to C_{n-1}, where n is the number of carbon atoms in the hydrocarbon. *n*-Octane formed, in addition, a small amount of octanol. These data would indicate that attack of a paraffin by oxygen under these conditions is not at the terminal carbon, but at the carbon in the CH_2 group adjacent to the terminal CH_3 group. This is consistent with the views of Burwell (3), who states that, whereas at high temperatures terminal carbons are attacked to produce aldehydes, at lower temperatures (*ca.* 150°C.) the beta carbon is involved primarily, the gamma secondarily, and so on toward the center of the molecule. Thus, formic, acetic, and propionic acids would be expected in order of decreasing concentration. Fenske *et al.* (19, 20) actually found this to be the case in an analysis of the volatile products resulting from the oxidation of a lubricating oil at temperatures of 130–180°C.

George, Rideal, and Robertson (22) found that C_{15} and C_{25} paraffins and alkylbenzenes are oxidized at 100–120°C. to form hydroperoxides, which accounted for 60–80 per cent of the oxygen absorbed by the alkylbenzenes, and which decomposed almost exclusively to give ketones in the case of the paraffins. The presence of peroxides in high concentration in the early stages of oxidation has also been shown by Larsen and coworkers (31). For example, in the oxidation of decalin at 110°C. peroxide is the principal oxidation product until upwards of 5000 cc. of oxygen per 100 g. of hydrocarbon has been absorbed. Balsbaugh and Oncley (2) found that tetralin, decalin, and cetane are oxidized almost exclusively to peroxides in the early stages of the reaction at temperatures ranging from 30° to 100°C. Similar results have been reported by Dornte (14) and by Denison (13) regarding the oxidation of white oils. Several investigators have shown that water is one of the principal oxidation products of lubricating oils (14, 19, 31, 32). Fenske and coworkers (19) report that water can account for as much as 44 to 70 per cent of the oxygen absorbed by an oil. In view of the above, the mechanism of the oxidation of a paraffin at temperatures in the range of 100–200°C. can be postulated as follows:



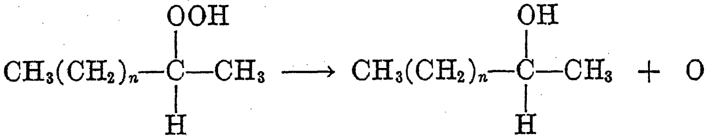
$$O_2 + CH_3(CH_2)_n-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{H}{C}}-H \rightarrow CH_3(CH_2)_n-\overset{O-O-H}{\underset{H}{C}}-CH_3 \rightarrow CH_3(CH_2)_nCOCH_3 + H_2O$$

Paraffin Hydroperoxide Ketone

$$O_2 + CH_3(CH_2)_nCOCH_3 \rightarrow CH_3(CH_2)_nCOOH + HCHO$$

Ketone Carboxylic acid Formaldehyde

In addition, there would be some primary attack on the gamma rather than the beta carbon atom. This would result in the formation of a ketone of the same number of carbon atoms, but with the C=O group shifted one carbon atom toward the center of the molecule. The corresponding carboxylic acid would contain one less carbon atom than shown above, and acetaldehyde, rather than formaldehyde, would be split off. The acids can be oxidized further through attack at other carbon atoms, and the aldehydes are probably oxidized further to the corresponding carboxylic acids and, to some extent, to carbon dioxide and water. The formation of alcohols would require a reducing action on a hydroperoxide, the latter losing one atom of oxygen in acting as an oxidizing agent. Esters could be formed by the condensation of an acid and an alcohol.

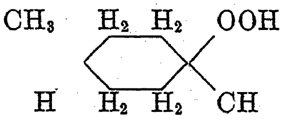


$$CH_3(CH_2)_n{-}\underset{\displaystyle H}{\overset{\displaystyle OOH}{C}}{-}CH_3 \longrightarrow CH_3(CH_2)_n{-}\underset{\displaystyle H}{\overset{\displaystyle OH}{C}}{-}CH_3 + O$$

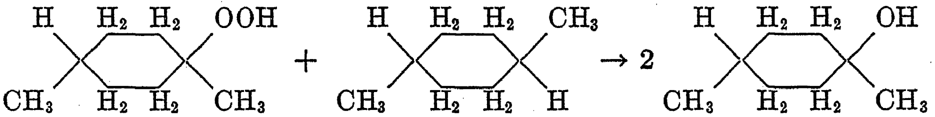
Hydroperoxide Alcohol

Chavanne and Bode (4) have made an extensive study of the oxidation products of 1,4-dimethylcyclohexane. Conditions were the same as in the case of the oxidation of the three paraffins mentioned above, except that the temperature was 100°C. instead of 120°C. They started with 116 g. of hydrocarbon and recovered 21 g. of unreacted material, so that 95 g. was oxidized. The oxygen consumed amounted to 30.7 g., and 123 g. of oxidation products as recovered, as compared with a theoretical of 95 + 30.7, or 125.7 g. The principal oxidation product was 1,4-dimethylcyclohexanol, which was present in sufficient quantity so that 30 g. was recovered in pure form. Other products included water (amount not given), carbon dioxide (5.2 g.), β-methyl-δ-acetylvaleric acid (8–9 g.), acetic acid (4–5 g.), β-methylvaleric acid (2–3 g.), dimethylcyclohexanediol (5.5 g.), and acetonylacetone (0.5 g.). Small amounts of hydrogen, carbon monoxide, methane, ethane, and formic acid were also detected.

The authors postulated the formation of a hydroperoxide,

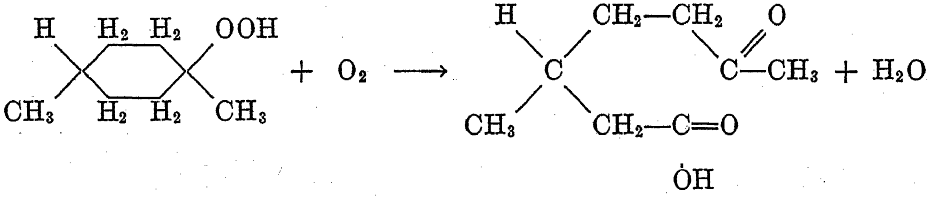


as the first step in the oxidation of 1,4-dimethylcyclohexane. In that respect, the mechanism would be identical with that proposed earlier in this paper for the oxidation of a paraffin, except that a tertiary rather than a secondary carbon atom is the vulnerable point of the molecule. The next step differs somewhat, however. The paraffin peroxide apparently decomposes largely through dehydration to give the corresponding ketone and, to a lesser extent, by reduction to the alcohol. The naphthene peroxide, on the other hand, apparently decomposes largely through reduction to the alcohol. This may be due in part to the fact that the tertiary carbon atoms in this particular hydrocarbon act as reducing agents for the peroxide, according to the following equation:



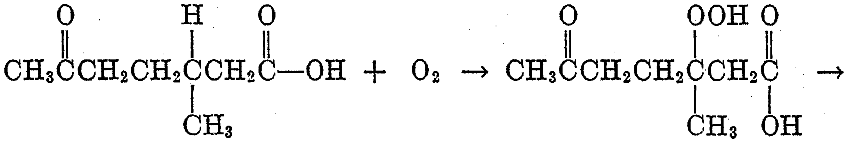
Hydroperoxide of 1,4-dimethylcyclohexane   1,4-Dimethylcyclohexane   1,4-Dimethylcyclohexanol

The formation of the diol can be explained in the same manner. The other oxidation products no doubt form as a result of further oxidation of the hydroperoxide, resulting in rupture of the naphthene ring. This mechanism necessarily differs from that involved in the paraffin in that a ketone cannot be formed, except by rupture of a C—C bond, for a tertiary carbon atom is involved. Thus the peroxide itself, and not the ketone, is oxidized further. Another difference is to be found in the oxidation products. The paraffin oxidizes to give first a peroxide, then a ketone, then an acid plus a "volatile oxidation product," —namely, formaldehyde or acetaldehyde or the corresponding acid. The naphthene peroxide can be oxidized with a rupture of a C—C bond and still form a product containing the original number of carbon atoms, as follows:



Hydroperoxide of 1,4-dimethylcyclohexane   β-Methyl-δ-acetylvaleric acid

Further attack of this molecule at the other tertiary carbon atom would explain the observed formation of acetonylacetone, as indicated by the following mechanism:



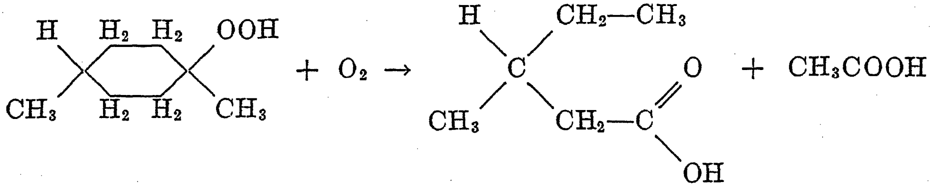
$$CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2\underset{\underset{CH_3}{|}}{\overset{\overset{H}{|}}{C}}CH_2\overset{\overset{O}{\|}}{C}—OH + O_2 \rightarrow CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2\underset{\underset{CH_3}{|}}{\overset{\overset{OOH}{|}}{C}}CH_2\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{C}} \rightarrow$$

β-Methyl-δ-acetylvaleric acid   Hydroperoxide of β-methyl-δ-acetylvaleric acid

$CH_3COCH_2CH_2COCH_3 + CH_2OHCOOH$

Acetonylacetone   Glycolic acid

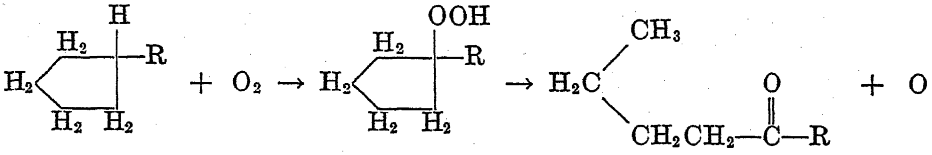
Glycolic acid was not reported by the authors. It may have been present and escaped detection, or it may have oxidized further to simpler end products like formic acid and carbon dioxide and water. The formation of β-methylvaleric acid can be explained by the further oxidation of the hydroperoxide of the hydrocarbon with two molecules of oxygen, and a split of the naphthene ring on either side of the tertiary carbon. This would result in the formation of one molecule of acetic acid and one of β-methylvaleric acid.



1,4-Dimethylcyclohexane hydroperoxide
β-Methylvaleric acid
Acetic acid

This does not explain the formation of hydrogen, methane, and ethane, which are probably formed through cracking. However, since these substances are formed only in very low concentration in comparison with the other products, their presence does not invalidate the reaction mechanisms as outlined above.

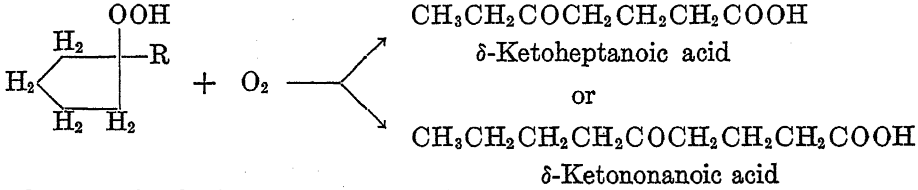
Dupont and Chavanne (17) studied the oxidation of the three cyclopentane derivatives ethyl-, butyl-, and phenyl-cyclopentane, again under conditions of slow oxidation. In every case they obtained a straight-chain ketone containing the original number of carbon atoms. These were ethyl *n*-butyl ketone, an unidentified nine-carbon-atom ketone which we shall assume to be dibutyl ketone, and phenyl butyl ketone, respectively. These three naphthenes probably formed hydroperoxides at the tertiary carbon atom in the same way that dimethylcyclohexane was oxidized; however, the peroxide then decomposed principally by reduction to the aliphatic ketone rather than to the cyclic alcohol as in the case of the cyclohexane derivative.



Ethyl-, butyl-, or phenyl-cyclopentane
Hydroperoxide
Ethyl butyl, dibutyl, or phenyl butyl ketone

Thus the end product resembles that formed from a paraffin, the only difference being that the paraffin hydroperoxide decomposes to the ketone by dehydration, losing one molecule of water, while the naphthene does so by reduction, losing one atom of active oxygen. The reason for the difference in the behavior of the C_5 and C_6 naphthenes is not clear. It could have been caused by the presence of two tertiary groups in the latter and only one in the former, or by the difference in size of either the ring or the side chains, or possibly to a difference in conditions.

Ethyl- and butyl-cyclopentanes also formed the δ-keto derivatives of heptanoic and nonanoic acids, respectively. These were probably formed by oxidation of the peroxide in the same manner in which β-methyl-δ-acetylvaleric acid was produced from dimethylcyclohexane.



$CH_3CH_2COCH_2CH_2CH_2COOH$

δ-Ketoheptanoic acid

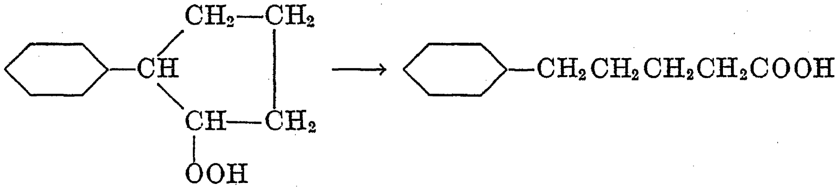
or

$CH_3CH_2CH_2CH_2COCH_2CH_2CH_2COOH$

δ-Ketononanoic acid

Hydroperoxide of ethyl- or butyl-cyclopentane

The former cyclopentane derivative also produced formic and propionic acids and the latter, butyric and valeric acids as products of further oxidation. Phenylcyclopentane also produced δ-phenylvaleric acid and benzyl alcohol. The formation of the former is of particular interest. This compound has the same empirical formula as the hydroperoxide of phenylcyclopentane; hence the acid could conceivably be formed from the peroxide by a molecular rearrangement. The peroxide in this case would probably not be the one with oxygen attached to the tertiary atom, but the one with oxygen attached to the naphthene carbon adjacent to it. The rearrangement would then include a break of a C—C bond, the transfer of a hydrogen atom from one carbon to another, and the transfer of an OH group from oxygen to carbon. Benzyl alcohol is possibly a degradation product of this acid.



Hydroperoxide δ-Phenylvaleric acid

Stevens (41) and Stevens and Roduta (42) have made an interesting study of the slow oxidation of a series of benzene derivatives. They bubbled oxygen through the hydrocarbon for a number of days at temperatures ranging from 80–140°C. and analyzed the products formed. Their data are summarized in table 1. It will be seen that oxidation always centers about a carbon atom attached to the benzene ring. The methylbenzenes all formed aldehydes containing the same number of carbon atoms as the parent molecule, and the substituted benzenes containing larger groups all formed ketones containing a phenyl group plus the other R group originally attached to the benzene ring, or the smaller of the two groups in the case where two groups were present. Acids, resulting from the further oxidation of aldehydes or ketones, were also formed, as well as acids of low molecular weight which formed from the fragments split off in the formation of ketones from secondary compounds. Alcohols were not formed in detectable quantity in any instance.

While Stevens does not report the presence of peroxides, it would appear probable that oxidation in every case was initiated by peroxidation to the hydroperoxide at the carbon adjacent to the benzene ring. This peroxide then decomposes by dehydration to the aldehyde or ketone, respectively, depending upon whether the starting material contained methyl or larger groups, or by the loss of a molecule of alcohol to form a ketone in the case of a tertiary hydrocarbon. The aldehydes may then be further oxidized to acids, and the alcohols to aldehydes and then to acids. The ketones are oxidized further to benzoic acid, probably by the same mechanism proposed earlier for the oxidation of a paraffin, to the acid of lower number of carbon atoms *via* the hydroperoxide and

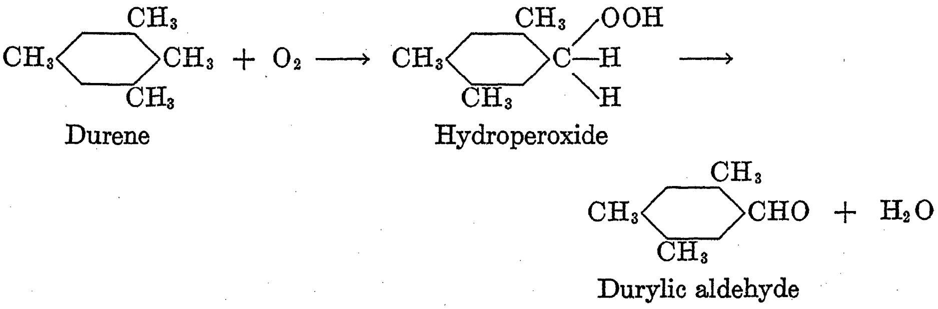
TABLE 1

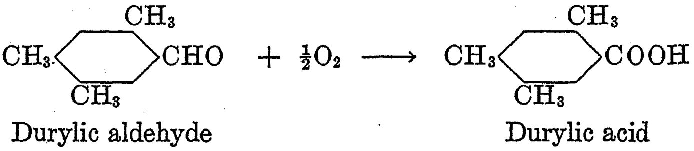
Oxidation of benzene aromatics

Data from Stevens (41) and Stevens and Roduta (42)

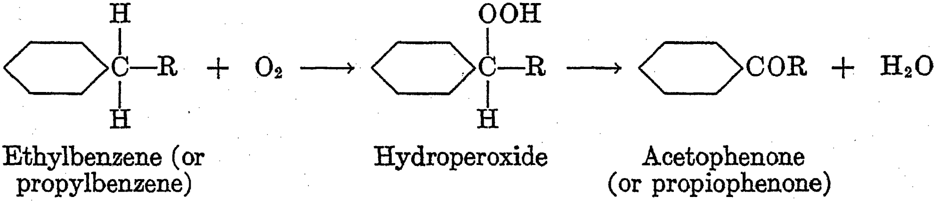
HYDROCARBON	OXIDATION TEMPERATURE	CONDITIONS (TIME)	GRAMS OF PRODUCT PER 100 G. OF HYDROCARBON
	°C.	*hours*	
Toluene	100	48	Trace of aldehyde
m-Xylene	100	30	2.1 g. toluic aldehyde; 1.1 g. toluic acid
Mesitylene (1,3,5-trimethylbenzene)	100	24	2.1 g. 1,3-dimethylbenzaldehyde; 1.4 g. mesitylenic acid
Durene (1,2,4,5-tetramethylbenzene)	100	9	5.8 g. durylic aldehyde; 4.9 g. durylic acid
p-Cymene (1-methyl-4-isopropylbenzene)	85	14	1.2 g. cumic aldehyde; 0.8 g. cumic acid
	102–4	14	Cumic aldehyde; cumic acid; 2 g. *p*-tolyl methyl ketone; formic acid
Ethylbenzene	110–15	24	19 g. acetophenone
n-Propylbenzene	102–4	25	Resin
	78	36	Trace of propiophenone
Cumene	102–4	23	4 g. acetophenone
(isopropylbenzene)	80	32	Formic acid
tert-Butylbenzene	102–4	25	Trace of non-volatile residue
Ethylmethylphenylmethane	119	14–29	2–10 g. acetophenone
	140	90	7.5 g. acetophenone; 4.2 g. benzoic acid
n-Butylmethylphenylmethane	119	14–29	2–10 g. acetophenone; butyric acid identified by odor
Methyldiphenylmethane	119	14–29	2–10 g. benzophenone; formic acid
Triphenylmethane	119	14–29	2–10 g. benzophenone; phenol
Diphenylmethane	119	14–29	2–10 g. benzophenone

ketone. These reactions, which would explain all of the products Stevens reports, are illustrated below:


Durene
Hydroperoxide
Durylic aldehyde


Durylic aldehyde
Durylic acid

The reactions of toluene, xylene, mesitylene, and *p*-cymene may be written in exactly the same manner, the products in every case being aldehydes and acids containing the same structure in the carbon skeleton as the original hydrocarbon. The reaction for ethylbenzene (and *n*-propylbenzene) is represented as follows:


Ethylbenzene (or propylbenzene)
Hydroperoxide
Acetophenone (or propiophenone)

$$C_6H_5COR + O_2 \longrightarrow C_6H_5COOH + R'CHO$$

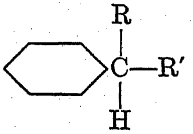
Acetophenone (or propiophenone) — Benzoic acid — Formaldehyde (or acetaldehyde)

$$R'CHO + \tfrac{1}{2}O_2 \longrightarrow R'COOH$$

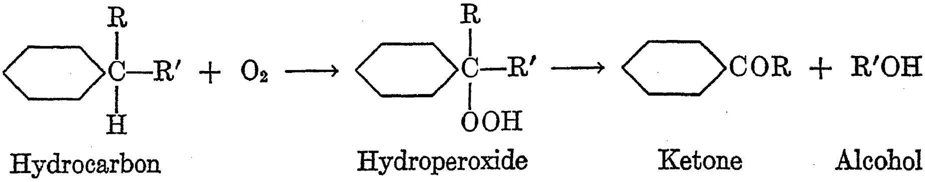
Formaldehyde (or acetaldehyde) — Formic acid (or acetic acid)

Benzophenone could be formed from diphenylmethane by the same mechanism shown for the oxidation of ethylbenzene to acetophenone.

Cumene, ethylmethylphenylmethane, methylphenylpropylmethane, butylmethylphenylmethane, methyldiphenylmethane, and triphenylmethane can all be represented by the structural formula,



where R and R′ are H or alkyl or phenyl groups which may be alike or different. R is the smaller of the two in cases of unequal size. *p*-Cymene may also be thought of as belonging to this group, although it contains an additional methyl group on the benzene ring. These hydrocarbons are probably oxidized according to the following scheme:



$$\underset{\text{Alcohol}}{R'OH} + \tfrac{1}{2}O_2 \longrightarrow \underset{\text{Acid}}{R''COOH}$$

tert-Butylbenzene cannot form a hydroperoxide at the carbon adjacent to the benzene ring, since this is a quaternary carbon. This may account for the fact that very little oxidation of this compound took place under conditions comparable to those for the other hydrocarbons.

Chernozhukov and Krein (8) studied the oxidation products of a number of hydrocarbons, although they did not report the oxidation products in detail as did the previously reported investigators. Their technique differed somewhat in that they used a bomb, and oxygen or air at a pressure of 15 atm. They used temperatures of 110–150°C. for the most part, which are comparable to those used by Chavanne and by Stevens. They found that substituted naphthenes are more unstable than unsubstituted ones, and attribute this to oxidation at the carbon of the ring attached to the alkyl group. They point out that this behavior—namely, the splitting of the ring—is in marked contrast to that of aromatic rings, which remain intact. Both of these observations are in line with the mechanisms proposed above for the initial oxidation of naphthenes and aromatics. They found that aromatics in general tend to give condensation products to a much greater extent than do naphthenes.

Larsen, Thorpe, and Armfield (32) studied the rate of oxygen absorption of a number of hydrocarbons at temperatures of 110–150°C. and at an oxygen pressure of 1 atm. While they did not analyze the products for specific compounds, they did report various functional groups. Thirty-nine compounds were investigated. These are tabulated in the section on "Oxidation rates." The average values for the products of oxidation of five classes of compounds are given in table 2. Conditions of oxidation were 1 atm. of oxygen and 110°C., except in the case of the less reactive naphthalene compounds, in which case the temperature was raised to 150°C. The total amount of oxygen absorbed varied from less than 100 cc. to more than 10,000 cc. per mole. In most cases it was of the order of 5000 cc. per mole.

The figures in table 2 cannot be regarded as absolute, since the total of the various products for a given hydrocarbon varied from 53 to 157 per cent of the total oxygen absorbed. However, in most cases these totals lay between the limits of 80 to 120 per cent, and the data shown in table 2 are probably quite accurate as regards the average distribution of oxygen. Peroxides do not constitute a major oxidation product; however, Larsen *et al.* have shown elsewhere (31) that peroxide content rises sharply in the early stages of oxidation and then falls. The free acid, alcohol, carbonyl, water, and volatile acid can all be explained as decomposition products and further oxidation products of the peroxides, and the combined acids, as determined by saponification value, by condensation reactions.

No value is listed for the average value under "volatile acids" for paraffins, since this value was reported for only one paraffin, hydropolyisobutylene. The value of 6.0 per cent reported is considered significant, in view of the fact that it is so much higher than those for the other hydrocarbons. It will be recalled that earlier in this paper it was pointed out that, according to the proposed theory, a paraffin can be oxidized to an acid only through the splitting off of a C_1, or sometimes C_2, or larger, fragment, which would appear as aldehyde or acid. Polyisobutylene also showed a relatively high percentage of oxygen absorbed as "volatile acid" (6.3 per cent), although the other olefin examined, tetraisobutylene, gave only 0.8 per cent.

TABLE 2

Oxidation products of five classes of hydrocarbons

Data taken from Larsen, Thorpe, and Armfield (32); all values expressed as percentage of total oxygen consumed

CLASS OF HYDROCARBON	FREE ACID	COMBINED ACID (ESTER)	PEROXIDE	ALCOHOL AND PHENOL	CARBONYL	H_2O	CO_2	VOLATILE ACIDS
Paraffin	14.3	16.3	4.1	1.9	46.0	43.9	4.7	
Naphthene and alkylnaphthene	11.2	17.0	13.5	8.9	51.4	21.9	3.8	0.6
Aromatic naphthene	6.1	23.1	4.3	8.5	27.2	16.7	1.2	0.4
Alkylbenzene	9.5	12.7	6.7	3.3	36.3	18.2	6.5	Trace
Naphthalene and alkylnaphthalene	6.9	16.3	1.4	9.4	9.6	51.3	7.8	1.6

Larsen *et al.* (31) found, as did Chernozhukov and Krein, that aromatics give condensation products which darken the oil and precipitate, whereas paraffins and naphthenes remain homogeneous and light in color upon oxidation.

B. Mixtures and petroleum fractions

Larsen *et al.* (31) also determined functional groups in the oxidation products of a number of lubricating oils. Oxidation was carried out in the same equipment and under the same conditions used in the study of pure hydrocarbons—namely, 1 atm. of oxygen and 110° or 150°C. The results for nine oils are shown in table 3. The first seven are lubricating oil fractions, while the eighth is a synthetic oil made by polymerization of cracked-wax olefins. The last is a white oil. Both of the last two are aromatic-free. The first six are California furfural raffinates of various stages of extraction, as indicated by the progressively increasing viscosity index (V.I.). It will be observed that as aromatics are removed there is a marked tendency toward a lower asphaltene content in the oxidized oil. This is of course one of the main purposes of solvent extraction, and is consistent with the conclusion based upon work on pure hydrocarbons that aromatics give dark insoluble condensation products. Extraction also results in a higher conversion of oxygen to water, and slightly less to carbon dioxide and to volatile acids. No trends are apparent in the free or combined acids, nor in the alcohols and carbonyl. No peroxides were found in the oxidation products of the California oils, indicating that any peroxides formed were unstable under these conditions, and immediately reacted further. The two aromatic-free oils formed no asphaltenes, and formed less carbon dioxide and water and more intermediate oxidation products. This was particularly true at the lower temperature. Comparison of results on the synthetic oil at the two temperatures shows that the higher temperature favors the decomposition of peroxides and carbonyl, and the formation of carbon dioxide and water.

TABLE 3

Oxidation of lubricating oils

Data from Larsen *et al.* (31); all values expressed as percentage of total oxygen consumed

OIL	TEMPERATURE OF OXIDATION	O_2 ABSORBED	FREE ACID	COMBINED ACID	PEROXIDE	ALCOHOL	CARBONYL	H_2O	CO_2	VOLATILE ACIDS	ASPHALTENE (MG. PER 10 G. OIL)
	°C.	*cc. per 100 g.*									
40 V.I.*California	150	2826	5.0	11		2.9	1.5	36	21	2.4	135
50 V.I. California	150	3010	4.5	10	0	8.9	8.9	51	26	2.0	100
60 V.I. California	150	2853	4.7	12	0	2.5	1.4	56	12	1.7	65
70 V.I. California	150	2800	4.1	9.0	0	.4	1.2	68	9.2	1.6	25
80 V.I. California	150	2803	3.8	10	0	.6		60	16	1.4	6
85 V.I. California	150	2800	4.1	12		1.3	2.5	65	16	1.2	2
Mid-Continent neutral	150	3233	4.5	12	<.1	0.7	5	49		1.1	
Synthetic oil made from cracked wax olefins	150	2532	5.9	16	<1	9	9	34	7	2.4	0
	110	2980	6.6	15	.4	8	19	26	4.9	3.8	0
White oil	110	1571	13	19	5.0	13	44	11		1.3	0

* V.I. refers to viscosity index as defined by Dean and Davis.

A comparison of the data on the lubricating oils as a whole with table 2 reveals that the former correspond most closely to the alkylnaphthalenes in their oxidation products. This would indicate that this class of compounds may be the "front line" which bears the brunt of the attack of a lubricating oil by oxygen. This phase of the subject will be discussed in greater detail in a later section of this paper.

Dornte and coworkers (14, 15, 16) studied the oxygen absorption of a number of oils, and found with a white oil at 135°C. that peroxides were the principal oxidation products. Carbonyl was next in order, with water and acids next. Only a trace of carbon dioxide was found. With lubricating oils Dornte used a temperature of 175°C. and found that about 40 per cent of the oxygen was converted to water, and 8–10 per cent to carbon dioxide.

Fenske *et al.* (14, 20), who used a similar apparatus and technique, found that Pennsylvania lubricating oils are oxidized at 170°C. to give the following distribution of oxygen in the oxidation products: water, 44–70 per cent; carbon dioxide, 3–9 per cent; carbon monoxide, 0.6–3.2 per cent; volatile acids, 1–7.5 per cent; fixed acids, 2–2.5 per cent; and 2–7 per cent isopentane insolubles, assuming that the latter contain 15 per cent oxygen. They found that the distribution of oxygen is influenced little by temperature over the range of 150–180°C., provided the amount of oxygen consumed is held constant, but that it varies as oxidation proceeds.

Hicks-Bruun, Ritz, Ledley, and Bruun (26) made similar studies. They worked at a temperature of 175°C. and found that 34.5–43.5 per cent of the oxygen could be accounted for as water, and 3.1–7.5 per cent as carbon dioxide. They compared an oil containing 19 per cent aromatic rings by Waterman analysis with one containing 3 per cent, and found that the former produced three to four times as much "soluble sludge" and "insoluble sludge" as the latter. This was of course to be expected, in view of the effect of aromatics as discussed earlier. They also found that the less aromatic oil formed more water and less carbon dioxide than the more aromatic sample. This again is consistent with the trend shown in table 3.

Davis, Lincoln, Byrkit, and Jones (12) studied the oxidation of lubricating oils by measuring the pressure drop in a Sligh flask containing the sample of oil and oxygen, and fitted with a manometer. They showed that the oxidation products remaining in solution, after precipitation of the asphaltenes by the addition of naphtha, can be removed by adsorption, from naphtha solution, on a suitable clay. These oxidation products, which can be desorbed from the clay by means of a polar solvent such as acetone or alcohol, are termed resins. The de-resined oil, after removal of the naphtha, has virtually the physical properties of the original unoxidized oil. This means of separation suggests the use of the resin content of a used oil as a good criterion of extent of oxidation, since it includes a large number of classes of oxidation products. Such an application is, however, complicated by the fact that there is no sharp line of demarcation between oil and resins or between resins and asphaltenes, but a more or less uniform series ranging from the least polar unoxidized hydrocarbon to the most insoluble asphaltene.

The oxidation of paraffin wax and of paraffinic oil fractions has received extensive study, largely by Russian investigators (11, 33, 35–38, 40, 43–45), for the purpose of producing fatty acids from petroleum. While the goal in such work is diametrically opposed to that of lubricating oil problems in that the purpose is to promote rather than stifle oxidation, the conditions used are comparable to those met by lubricating oils, and the products formed are of interest in the present survey.

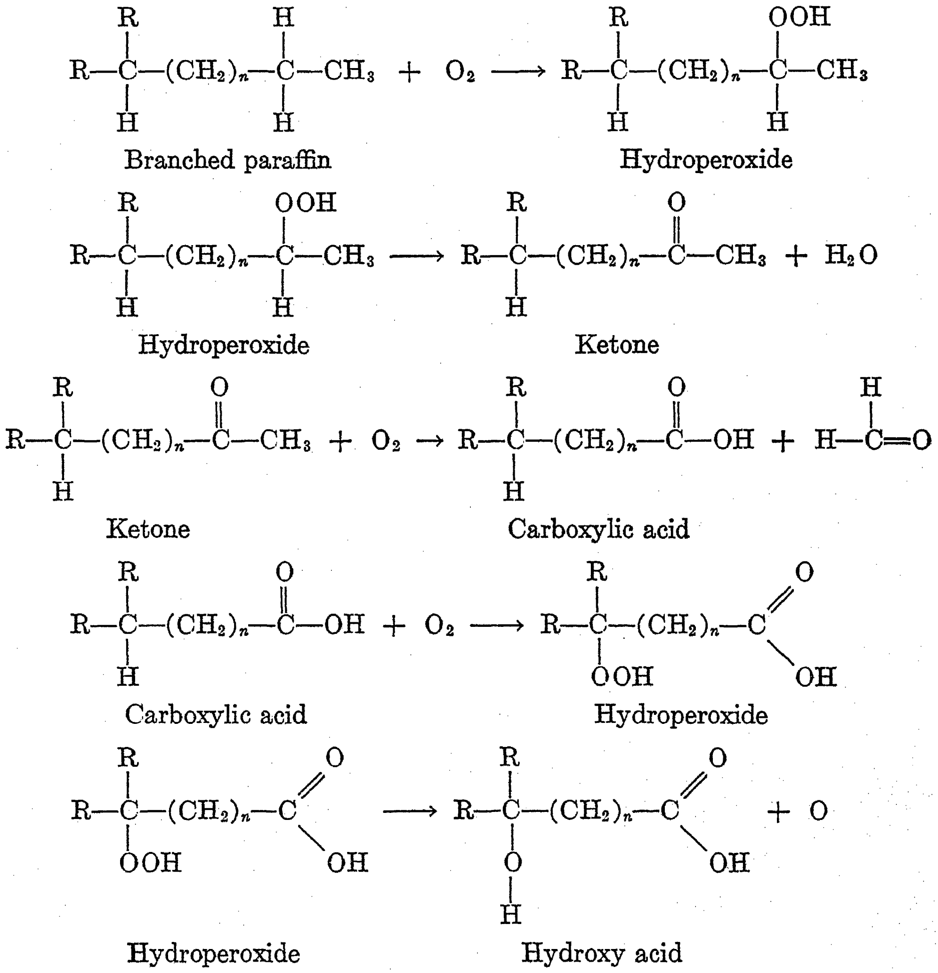
Likhuskin, Masumyan, and Levkupulo (33) state that the oxidation of a wide fraction of fuel oil with air at 130°C. will give yields as high as 41 per cent of acids. Higher yields are accompanied by excessive quantities of hydroxy acids. Sodium naphthenate was used as a catalyst. Danilovich and Dianina (11) prefer oxidation in four to five stages, with calcium naphthenate as catalyst, and report water-white fatty acids as products. Varlamov (43) studied the oxidation of Grozny paraffin at temperatures of 160–180°C. with air at 15–30 atm. pressure. From 20 to 74 per cent of the paraffin reacted, and 70 per cent or more of the oxidation products consisted of fatty acids of varying solubility. He found low-molecular-weight alcohols, aldehydes, and ketones in the volatile oxidation products. He also conducted some experiments in the absence of water, to determine whether this would influence the formation of hydroxy acids. He found, contrary to his expectations, that a high yield of acids could be obtained in the absence of moisture, and that a high percentage of the products consisted of hydroxy acids. He found manganese, copper, calcium, and nickel to be positive catalysts. Velikovskiĭ and Lemer (44) oxidized paraffin and slack wax with air at a temperature of 160°C. both in iron and in aluminum equipment. They found that the former metal favored the formation of insoluble hydroxy acids, particularly in the case of slack wax. With stepwise oxidation they were able to obtain maximum yields of acids, and a minimum of hydroxy acids. Velikovskiĭ and Vasil'eva (45) investigated the oxidation of various crude petroleum fractions, and found that in general reaction cannot be effected without a catalyst, but that oxidation could be accomplished with the aid of catalysts or by pretreatment of the oil with oleum. Calcium oleate was found to be an effective catalyst, and manganese oleate even more so. Petrov (35) describes a commercial process in which a petroleum distillate is sulfonated and the unsulfonated residue oxidized at 96–115°C. in the presence of a calcium or manganese soap until a 20 per cent yield of fatty acid is reached.

Plisov, Galandeev, and Zel'tsburg (37) point out that the petroleum ether-insoluble hydroxy acids, which are generally regarded as undesirable by-products in the manufacture of fatty acids, are valuable as film-forming substances in the lacquer and pigment industries, and as raw materials in the plastics industry. By oxidizing with air at 115–120°C. they were able to obtain yields of 80–86 per cent of hydroxy acids, based upon total acids produced. They regard the product as a mixture of hydroxy acids, lactones, and lactides.

Shoruigin and Kreshkov (40) oxidized paraffin wax with air at 160°C. and found the following products: water, hydroxy acids, lactides, and carboxylic acids and their anhydrides. The following straight- and branched-chain compounds, presumably hydroxy acids, were found: $C_{10}H_{20}O_3$, $C_{13}H_{26}O_3$, $C_{14}H_{28}O_3$, $C_{15}H_{30}O_3$, $C_{17}H_{34}O_3$ (two isomers), $C_{18}H_{36}O_3$, $C_{21}H_{42}O_3$, (two isomers), $C_{22}H_{44}O_3$, $C_{25}H_{50}O_3$, $C_{27}H_{54}O_3$, and $C_{32}H_{64}O_3$. The presence of β-hydroxy acids was indicated.

Plisov (36) reports that Grozny paraffin, having a melting point of 52°C., is oxidized at 140–160°C. to form peroxides which are capable of liberating iodine from potassium iodide and of oxidizing ferrous salts, and which are stable to heat. In addition, they report peroxides which are also stable, but which differ in that they do not respond to the usual tests for peroxides. Both types of peroxide are decomposed by acid or alkali, and they undergo hydrolysis by water. The last-named reaction is catalyzed by calcium and sodium naphthenates.

The production of carboxylic acids from paraffin wax can be explained as outlined earlier in Section II,A: the paraffin is oxidized to the β-hydroperoxide, which decomposes to a methyl ketone, which in turn is oxidized further to formaldehyde plus a carboxylic acid containing one carbon less than the original paraffin. The formation of hydroxy acids may be associated with branched-chain paraffins. Although Shoruigin and Kreshkov found both straight- and branched-chain compounds of the empirical formula $C_nH_{2n}O_3$, the majority of work reported indicates that paraffin wax shows less tendency to undergo oxidation to the hydroxy acid than do oil fractions or slack wax. The oxidation of a branched paraffin to an hydroxy acid could proceed according to the following mechanism:



III. OXIDATION RATES

A. Pure hydrocarbons

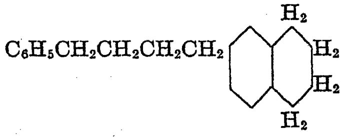
Larsen, Thorpe, and Armfield (32) have made an extensive study of the oxidation rates of a number of pure hydrocarbons. They measured oxygen absorption at 110°C., except in the case of the more stable compounds, which were oxidized at 150°C. Their results on thirty-nine hydrocarbons, representing paraffins, olefins, naphthenes, benzene and naphthalene aromatics, and combinations of these types in the same molecule, are summarized in table 4. For the sake of brevity the compounds are classified in this tabulation only according to the time required to absorb 2000 cc. of oxygen per gram-mole and according to the type of curve obtained. (It should be understood that the "type of curve" applies only under the given set of conditions. von Fuchs and Diamond (21) have shown that a given oil may exhibit autocatalysis or autoretardation, depending upon the temperature of oxidation.) Two thousand cubic centimeters of oxygen per gram-mole corresponds to the oxidation of approximately 9 per cent of the molecules with one molecule of oxygen per molecule of hydrocarbon, or 4.5 per cent with two molecules of oxygen. It will be recalled that one molecule is required to convert a paraffin to a peroxide or ketone, and an additional molecule to convert the ketone to a carboxylic acid plus low-molecular-weight aldehyde. For a lubricating oil with a molecular weight of 400, the figure of 2000 cc. per gram-mole corresponds to 500 cc. per 100 g. The four types of curve are the autocatalytic, the autoretardant, the linear, and the combination type in which autocatalysis prevails first, then autoretardation. Table 4 lists the hydrocarbons in order of increasing stability. In comparing two compounds oxidized at different temperatures, a factor of 16 was used to convert the rate of oxidation at 110°C. to that at 150°C. This factor is based upon a doubling of reaction rate per 10°C. and is not strictly correct, but sufficiently close for the purpose of comparison.

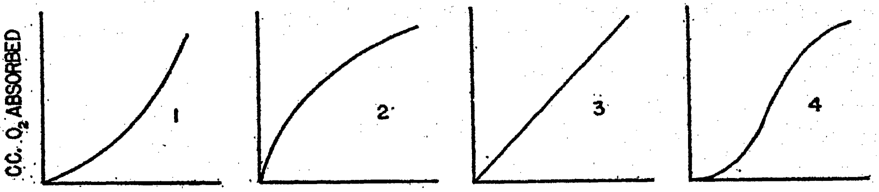
It will be observed that the ten paraffins, naphthenes, and alkylnaphthenes all had "2000 cc. times" between 12 and 47 hr. at 110°C., and all showed the autocatalytic type of curve, at least in the early stages (type 1 or 4). The most rapid oxidation was shown by hydrocarbons containing either a double bond, a partially hydrogenated condensed ring, or a benzene ring with a long paraffinic side chain. Surprisingly, these compounds, eleven in number, which had "2000 cc. times" ranging from 1.5 to 12 hr. at 110°C. do not in general give an autocatalytic type of oxidation curve. Only one gave a type 1 curve, and four a type 4. The other six were equally divided between types 2 and 3. Thus autocatalysis is not a requisite for rapid oxidation. The naphthalene derivatives were as a class the most stable, and most of their oxidation curves were of either the autoretardant or the linear type.

The effect of olefinic unsaturation is brought out by a comparison of items 7 and 13. The latter, hydropolyisobutylene, was obtained by hydrogenation of the former, polyisobutylene, which contained one C—C bond per C_{13} molecule. The paraffin had a 2000 cc. time of 17 hr., which is more than four times that of the olefin.

Oxidation rates of pure hydrocarbons

Data from Larsen, Thorpe, and Armfield (32)

	HYDROCARBON	OXIDATION TEMPERATURE	TIME TO ABSORB 2000 CC. OF O_2 PER GRAM-MOLE	TYPE OF CURVE (SEE FIGURE 1)
		°C.	*hours*	
1	Tetralin	110	1.5	2
2	Tetraisobutylene	110	2	3
3	Octahydroanthracene	110	2	4
4	9,10-Dihydro-9,10-diisobutylanthracene	110	2.5	2
5	*n*-Octadecylbenzene	110	2.5	3
6	β-*n*-Octadecyltetralin	110	3.5	4
7	Polyisobutylene, $(C_4H_8)_{13}$	110	4	3
8	α-Phenyl-Δ²-tetralylbutane	110	7	4
	 $C_6H_5CH_2CH_2CH_2CH_2$ H_2 H_2 H_2 H_2			
9	9,10-Diisobutylperhydroanthracene	110	7	1
10	5-Isobutylacenaphthene	110	8	2
11	*n*-Hexadecylbenzene	110	12	4
12	Perhydroanthracene	110	12	1
13	Hydropolyisobutylene, $(C_4H_{10})_{13}$	110	17	4
14	Hexaethylbenzene	110	23	1
15	β-*n*-Octadecyldecalin	110	24	1
16	Fluorene	110	26	2
17	Decalin	110	27	1
18	Dicyclohexyl	110	28	1
19	*n*-Amylcylopentane	110	28	1
20	*n*-Amylbenzene	110	28	3
21	1-α-Naphthyl-1-*n*-butylhexadecene	150	2	3
22	*n*-Octadecylcyclohexane	110	37	1
23	*n*-Hexadecylcyclohexane	110	45	1
24	Cetane	110	45	1
25	*n*-Decane	110	47	1
26	*sec*-Amylbenzene	110	68	1
27	*tert*-Amylbenzene	110	85	1
28	Benzylnaphthalene	110	120	3
29	Diphenylmethane	110	>70	2
30	Di-α-naphthylmethane	150	12	1
31	α-*n*-Octadecylnaphthalene	150	33	1
32	Poly-*sec*-amylnaphthalene	150	42	1
33	α-Isoamylnaphthalene	150	55	2
34	α-Methylnaphthalene	150	62	2
35	Di-*sec*-amylnaphthalene	150	72	3
36	β-*sec*-Amylnaphthalene	150	140	1
37	β-Methylnaphthalene	150	>150	3
38	*tert*-Butylnaphthalene	150	>150	3
39	Naphthalene	150	>150	3



Hydrogenation of an alkylbenzene to the corresponding cyclohexane derivative resulted in an increase in stability, as shown by comparing item 5 with 22, and 11 with 23. The effect was to increase the 2000 cc. time by factors of 15 and 4, respectively, which are of the same order as that found in the case of the olefin. Both of these alkylbenzenes had long side chains (C_{16} and C_{18}), and the effect of hydrogenating benzene itself or a low-molecular-weight homolog would probably be quite different.

Hydrogenation of a naphthalene ring, on the other hand, results in a marked loss of stability, particularly if the naphthalene ring is only partially hydrogenated. The most stable hydrocarbon listed in table 4 is naphthalene, with a 2000 cc. time of > 150 hr. at 150°C. or > 2400 hr. at 110°C., assuming the above-mentioned factor of 16 for the difference in temperature of 40°C. Tetrahydronaphthalene, or tetralin, is on the other hand the least stable compound shown in table 4. It has a 2000 cc. time at 110°C. of only 1.5 hr. Thus the partial hydrogenation of naphthalene reduced its stability by a factor of more than 1600. Complete hydrogenation to decahydronaphthalene, or decalin, brings the 2000 cc. time back to 27 hr., which is 18 times that of tetralin, but still short of that of naphthalene by a factor of > 89. That chain length does affect the influence of hydrogenation of the naphthalene ring is shown by comparing the above results with a comparison of items 31, 15, and 6, which represent the octadecyl derivatives of naphthalene, decalin, and tetralin, respectively, although unfortunately, for purposes of the present comparison, the first is the alpha derivative, and the other two beta. Octadecylnaphthalene had a 2000 cc. time at 150°C. of 33 hr., which would correspond to 530 hr. at 110°C. and the tetralin and decalin derivatives 2.5 and 24 hr., respectively, at 110°C. Thus the factors relating the 2000 cc. times of the naphthalene to that of the tetralin and decalin derivatives are 530/2.5 and 530/24, or 210 and 22, respectively. The differences between these values and the corresponding ones of >1600 and > 89 for the unsubstituted homologs are probably due primarily to the effect of the alkyl group rather than the difference in position.

Item 21, 1-α-naphthyl-1-*n*-butylhexadecene, is interesting in that it contains both a naphthalene group, which would classify it with the stable hydrocarbons, and olefinic unsaturation, which would classify it with the unstable compounds. The two effects seem to balance each other, with the result that this compound has an intermediate stability comparable to that of the paraffins and naphthenes.

Comparison of items 20, 26, and 27 is of interest in that these three compounds are primary, secondary, and tertiary amyl derivatives of benzene, respectively, and hence show the influence of the structure of the side chain. According to the mechanism proposed earlier for the oxidation of an alkylbenzene, the first of these three hydrocarbons would form the hydroperoxide at the alkyl carbon attached to the ring. The peroxide would then dehydrate to phenyl butyl ketone. The second would likewise form the peroxide, but, since there was only one hydrogen atom on the carbon in question initially, propyl alcohol, rather than water, would split off, and acetophenone would be the other product. Since this process involves the breaking of a C—C bond, whereas the first does not, it is not surprising that the 2000 cc. time for the primary compound is less than half that for the secondary (28 hr. compared to 68). Furthermore, since the tertiary compound cannot form a hydroperoxide at the carbon attached to the ring, but must oxidize at other positions, it would be expected to be the most stable of the three, and this was again the case, for the 2000 cc. time for *tert*-amylbenzene was 85 hr. A similar trend was noted by Stevens and Roduta (42), who found *tert*-butylbenzene much more resistant to oxidation than any of the other alkylbenzenes which were examined (see table 1). The extreme stability of *tert*-butylnaphthalene as compared to the other naphthalenes (see item 38, table 4) is also in line with this trend.

Larsen, Thorpe, and Armfield (32) attribute the extreme stability of naphthalene aromatics in comparison with benzene homologs to the difference in the effectiveness of their oxidation products as inhibitors (naphthols in the one case *versus* phenols in the other). To support this theory, they oxidized *n*-amylbenzene (item 20, table 4) to which had been added 9.8 per cent of pre-oxidized α-methylnaphthalene. The curve of the mixture was practically coincident with that for α-methylnaphthalene itself (item 34). This represents a thirty-five-fold increase in 2000 cc. time, assuming again a factor of 2 per 10°C. change in temperature.

Chernozhukov and Krein (8) did not determine rates of oxidation, since most of the compounds they studied were oxidized in a bomb for a fixed time. It is possible, however, to compare the behavior of various compounds under identical conditions and thus determine relative stability. They found that naphthalene, anthracene, and biphenyl, after 3 hr. at 150°C. and 15 atm. of oxygen, had zero saponification values, whereas α-methylnaphthalene, β-methylnaphthalene, and propylnaphthalene produced, under these same conditions, saponification values of 12, 16, and 49, respectively. From this the authors conclude that chainless polynuclear aromatics are very stable, that the introduction of side chains, particularly long side chains, decreases stability, and that the beta derivatives of naphthalene are more reactive than the alpha. They also found that the presence of an intermediate link of carbon atoms between two aromatic rings, as in diphenylmethane, lowers stability. All of these conclusions are in agreement with those of Larsen, Thorpe, and Armfield, except that regarding the relative stability of α- and β-methylnaphthalenes. Chernozhukov and Krein found, as did Larsen *et al.* (31), that benzene derivatives are less stable than naphthalene homologs, and that the higher alkyl compounds are less stable than those of lower molecular weight. For example, after oxidation at 110°C. and 15 atm. of oxygen for 3 hr., 1,3,5-trimethylbenzene had a saponification number of 11, whereas propyl-, nonyl-, decyl-, *p*-methyl-, isopropyl-, and *o*-methyl-isopropylbenzenes had saponification numbers ranging from 31 to 48 after oxidation under the same conditions.

Balsbaugh and Oncley (2) studied the oxygen absorption of tetralin, decalin, and cetane at 30°, 75°, and 100°C., respectively, and found these three hydrocarbons to rate in the order of increasing stability in which they are mentioned. This is the same order shown for these compounds in table 4.

Hock and Lang (27) found that cyclopentene is oxidized at a slower rate than cyclohexene, and hydrindene, which can be regarded as tetralin minus one CH_2 group, at a slower rate than tetralin; from these results they conclude that a five-membered ring is less readily oxidized than a six-membered ring. However, their conditions differed from those used by others quoted above in that they used oxygen plus ultraviolet light. Their results cannot, therefore, be compared with the others without reservation.

B. Mixtures and petroleum fractions

When two hydrocarbons of unequal stability are blended, an intermediate stability might be expected, with the component of lower stability being oxidized preferentially. Actually a blend may possess a stability considerably greater than that of either component, and the least stable component is not necessarily oxidized in preference to the more stable one. Thus the stability of a blend of a few hydrocarbons or a mixture of several is probably determined to a large extent by interaction phenomena among various molecules or radicals. This, of course, greatly complicates the problem.

It has been pointed out earlier in this paper that the oxidation products of a lubricating oil resemble those of substituted naphthalenes, and that this class of hydrocarbons may, therefore, be oxidized preferentially in a blend, even though by themselves such compounds are exceedingly stable. This point is brought out in greater detail in table 5, which shows the effect of the addition of diamylnaphthalene to a white oil upon the distribution of oxidation products. The average data for naphthenes and alkylnaphthenes, to which class a white oil would belong, and for naphthalene derivatives, including diamylnaphthalene, are repeated from table 2 for comparison.

Of the six oxidation products listed, three—namely, free acid, peroxide, and carbonyl—show appreciably lower values for the napththalene than for the naphthene, and one, water, behaves in the opposite manner. Alcohols and combined acids or esters are produced to about the same extent in both classes of compounds. It is significant that as the percentage of diamylnaphthalene in white oil is increased from 0 to 50 per cent the percentage of oxygen appearing as free acid, peroxide, and carbonyl decreases progressively, and the percentage appearing as water increases accordingly. It would appear that in blends of 25–50 per cent of diamylnaphthalene in white oil, the former is oxidized preferentially, even though white oil has a 2000 cc. time at 130°C. of about 5 hr., compared with 72 hr. at 150°C. for diamylnaphthalene, or 288 hr. at 130°C., which means that by itself, the white oil is fifty-eight times as reactive as diamylnaphthalene.

That a blend of two components may be more stable than either by itself is brought out clearly in an example cited by von Fuchs and Diamond (21), who measured the time required for the absorption of 1800 cc. of oxygen per 100 g. of oil for a motor oil containing varying quantities of bright stock aromatics. Oxidation was effected in the presence of iron as a catalyst. They found that the curve relating absorption time to concentration of added aromatics passed through a distinct maximum in the vicinity of 5 per cent, dropping off to absorption times less than half of the maximum at 0 and 10 per cent. Thus this blend has a definite "optimum aromaticity." The concept of optimum aromaticity is of great practical importance; it, along with the more conventional critieria of viscosity index and yield, determines the proper degree of solvent extraction to which a given stock should be subjected.

That lubricating oils possess an optimum aromaticity with respect to stability was also found by Fenske *et al.* (19), who separated a Pennsylvania oil, containing 9 per cent of aromatic rings, into 124 fractions by a combination of vacuum distillation and solvent extraction. They studied the rate of oxidation of seven of the fractions, ranging in aromaticity from 0 to 2 per cent and from 15 to 40 per cent aromatic rings, along with the original material. They used a temperature of 140°C. in a conventional oxygen-absorption apparatus, and found that none of the fractions examined was as stable as the original oil. Two fractions of zero aromaticity were the least stable, with one containing 2 per cent aromatic rings next, two of 37 and 40 per cent next, and two of 15 and 25 per cent the most stable, except for the original oil. That this was not caused by damage inflicted in the processing was proved by an oxidation test on a blend of all 124 fractions in the original proportions, which checked the run on the original sample.

TABLE 5

Effect of the addition of diamylnaphthalene to a white oil upon the distribution of oxidation products

Data from Larsen *et al.* (31); all figures expressed as percentage of total oxygen consumed

	FREE ACID	COMBINED ACID (ESTER)	PEROXIDE	ALCOHOL	CARBONYL	H_2O
Average naphthene or alkylnaphthene	11	17	14	8.9	51	22
White oil (mol. wt. = 387)	13	20	5	13	44	17
White oil + 12.5% diamylnaphthalene	16	22	2.6	5.1	33	21
White oil + 25% diamylnaphthalene	9	23	0.2	6.4	14	24
White oil + 50% diamylnaphthalene	5.7	28	0.3	8.0		57
Average naphthalene derivative	7	16	1.4	9.0	10	51

An optimum aromaticity for insulating oils is indicated in the work of Clark (9), who studied the dielectric stability (which is known to be closely related to oxidation stability) of a series of oils with varying olefinic and aromatic unsaturation. Olefinic unsaturation was determined by absorption in sulfuric and boric acids, and aromatic plus olefinic unsaturation by absorption in sulfuric acid and phosphorus pentoxide, both according to Kattwinkel. Clark found a sharp and progressive decrease in dielectric stability with increasing olefinic unsaturation over the range of 0–6 per cent; however, variation in aromatic unsaturation from 0–12 per cent showed a maximum stability in the vicinity of 6 per cent.

von Fuchs and Diamond (21) found that the shape of the rate curve can be drastically altered by the concentration of added aromatics and by temperature. This necessitated a revision of the theory proposed by Dornte *et al.* (14, 15, 16), who classified oils into three groups: those whose oxidation products were (*1*) positive catalysts, (*2*) negative catalysts, or (*3*) without any catalytic effect. von Fuchs and Diamond concluded that aromatics can possibly "act in a two-fold capacity—i.e. both as inhibitors and retardants; either several compounds present may be acting differently or the same compound may be playing a dual rôle. At low concentrations conventional inhibitor action outweighs the other and an induction period is observed; at higher concentrations this effect is obscured by the additional quantity of aromatics, and autoretardation predominates. At intermediate concentrations the opposing tendencies of autocatalysis and autoretardation are balanced against each other more evenly so that the rate curve may possess an intermediate curvature or even be linear."

Larsen, Thorpe, and Armfield (32) studied the effect of adding naphthalene derivatives to other substances, and found a stabilizing effect. A much greater effect was, however, obtained when the napthalene compound was preoxidized in the absence of the other component. They attribute these results to the fact that in the former case oxidation of the naphthalene compound and oxidation of the second component are competitive, whereas in the latter the oxidation products of the alkylnaphthalene, probably naphthols, are present as antioxidants throughout the oxidation of the blend.

The effect of added aromatics upon the oxidation of paraffins and naphthenes has also been investigated by Chernozhukov (6) and by Chernozhukov and Krein (7, 8), who regard the stability of mineral oils to be determined essentially by the character and quantity of the aromatics present. They found, for example, that the addition of 1–10 per cent of naphthalene, phenanthrene, or anthracene to a vaseline oil resulted in a marked improvement in stability, according to their oxygen bomb test. They show this to be due to a preferential oxidation of the aromatics, with the oxidation products acting as antioxidants. They obtained similar results, though to a lesser degree, with substituted aromatics. This order of effectiveness of the substituted and unsubstituted aromatics in increasing the stability of a blend is the opposite of that found by Larsen, Thorpe, and Armfield (32), who found naphthalene ineffective in altering the oxidation curve of decalin, whereas substituted naphthalenes are effective. It must be remembered, however, that the conditions used in the two laboratories were different.

Denison (13) does not concur with Chernozhukov and others in their views on the importance of aromatics in determining the stability of a mineral oil. He considers natural sulfur compounds, rather than the hydrocarbon composition, to be the important factor. His conclusions are based on oxygen-absorption measurements on three oils before and after desulfurization, which was effected by treatment with metallic sodium in the presence of hydrogen at a pressure of 200 psi and at a temperature of 500°F. His results are summarized in table 6.

Denison reasons that since desulfurization resulted in little change in the hydrocarbon content of the oil, as shown by specific dispersion and Waterman analysis, and by the fact that no olefinic unsaturation was indicated by bromine number determinations, the decrease in stability was due solely to the removal of sulfur, and that "natural sulfur compounds seem to be the agents responsible for the stability of straight mineral oils." However, a glance at table 6 shows that, if this is the case, the relationship between stability and sulfur content is not a simple function, for the Pennsylvania oil, which was lowest of the three in sulfur both before and after treatment, was in both cases intermediate in stability, while the California naphthenic, which was highest in sulfur, was the least stable of the three before treatment, and the most stable after. Furthermore, the process of desulfurization necessarily alters the hydrocarbon structure. The sulfur was presumably removed by decomposition, with the rest of the molecule remaining in the oil. Since 2–20 per cent of the original oil consisted of sulfur compounds, it is scarcely conceivable that the changes in hydrocarbon structure do not affect stability. It is possible that a small amount of olefinic unsaturation escaped detection in the bromine number determination, since this analytical method is known to be less reliable for complex molecules as found in a lubricating oil than for the simpler olefins for which the method was devised. There is, moreover, a possibility that some reaction between sodium and aromatic rings took place, with the subsequent formation of tetralin-type compounds. Gilman, in his *Organic Chemistry*, reports that naphthalene can be reduced to tetralin by the action of sodium in liquid ammonia solution. It is conceivable that a similar reaction occurred under the conditions used by Denison. While the extent of such reactions could not have been great, since specific dispersion was not affected appreciably, the presence of a small amount of tetralin or related compound would be expected to have a profound influence on stability. It will

TABLE 6

Effect of desulfurization upon stability and properties

Data from Denison (13)

OIL	TIME TO ABSORB 100 CC. O_2 PER 100 G.	SULFUR	SPECIFIC DISPERSION	PER CENT AROMATIC RINGS (WATERMAN)
	hours	*per cent*		
California naphthenic SAE 30	0.7	0.53	120	15
California naphthenic SAE 30, desulfurized	0.2	0.07	121	14
California paraffinic 400 neutralized	5.0	0.22	106	1
California paraffinic 400 neutralized, desulfurized	<0.1	0.06		1
Pennsylvania SAE 30	2.5	0.10	111	7
Pennsylvania SAE 30, desulfurized	0.1	0.01	110	4

be recalled from the discussion of table 4 that tetralin is more than 1600 times as reactive as naphthalene. Furthermore, tetralin forms copious quantities of peroxides (2) which probably act as proöxidants, whereas naphthalene forms naphthols which act as antioxidants (21).

In view of this analysis of the problem, it would appear that the relative importance of aromatics and sulfur compounds in determining the rate of oxidation of straight mineral oils cannot be stated definitely without further experimentation. Both are probably involved, rather than either one alone. In addition, compounds containing other elements, notably oxygen and nitrogen, and which occur naturally in the lubricating oil fraction of petroleum, undoubtedly play a rôle in determining the stability of an oil.

C. Effect of catalysts and inhibitors

All of the oxidation studies discussed thus far in this paper, with the exception of a few cases where catalysts were specifically mentioned, have been in the absence of added catalyst or antioxidant. In actual practice either of these classes of material can, and usually does, have a profound effect upon the rate of oxidation of a lubricating oil.

Catalysts for the oxidation of lubricating oil include a wide variety of compounds. Hanson and Egerton (24) report that nitrogen dioxide, in concentrations comparable to that present in engine cylinders, greatly influences the rate of oxygen absorption of an oil at 220°C., the effect being that of shortening the induction period. Oxidation products, notably peroxides, may themselves be catalysts, as evidenced by the concave-upward, or autocatalytic, type of oxygen-absorption curve exhibited by certain oils under certain conditions. However, these do not constitute the most serious source of catalysis of deterioration encountered by lubricating oils. Metal catalysis, principally by compounds of metals which can readily change valence and specifically by compounds of copper, lead, and iron, is always to be reckoned with. These three metals are probably no worse in their effect than several others which could act as catalysts, such as manganese, chromium, or vanadium, but they play a more important rôle because of their much greater prevalence: copper in the form of oil lines, brass fittings, and bearing metal; lead as bearing metal and as a constituent of the decomposition products of tetraethyllead; and iron as the major constituent of the engine itself.

Davis, Lincoln, Byrkit, and Jones (12) showed that the napthenates of iron, lead, copper, cadmium, and silver all act as catalysts for the oxidation of lubricating oil, the effect varying with concentration. They found that in general an oil can "tolerate" a certain amount of catalyst without any appreciable effect upon rate of oxidation, but that an increase in concentration beyond this threshold value results in a marked decrease in induction period. As the concentration is increased further, a point is eventually reached where the system is again insensitive to further change in concentration. For example, a Mid-Continent, vacuum-distilled, solvent-refined oil had an induction period of 80 min. at 175°C. The addition of iron naphthenate up to 0.006 per cent as Fe_2O_3 had no effect, but further addition to 0.1 per cent resulted in a linear decrease in induction period, on a log-log plot, to less than 20 min. Further addition of iron naphthenate was without noticeable effect. All of this work was done in a static system, and measurements were limited to the initial phases of oxidation, so that the conclusions reached need not necessarily apply under conditions of agitation of the oil and oxidation to a greater extent. It would appear, however, that oils contain natural "anticatalysts" which are capable of nullifying the effect of a certain amount of added catalyst, and that at high concentrations of metal soap the system reaches a state of catalytic saturation.

Fenske *et al.* (19), who used a conventional circulatory oxygen-absorption apparatus, studied the effect of copper, iron, and lead, both in metallic and in soluble naphthenate form. They found copper naphthenate to be the most potent of the three soaps, and lead the least. This same order prevailed for the bulk metals in the early stages of oxidation, but in the later stages, lead became the most potent of the three. This was attributed to the greater solubility of lead compounds in the oil, as compared with those of iron and copper. Fenske also found that catalysis by these metals has little effect in altering the distribution of oxidation products, the main effect being that of increasing the over-all rate of oxidation rather than that of any one specific reaction.

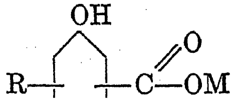
Larsen and Armfield (30) studied these same three catalysts, both in soluble and in bulk metal form, in three different oils, and at varying concentrations of soluble naphthenate and ratios of surface of metal to volume of oil. Their results with an extracted Mid-Continent oil were essentially in agreement with those reported by Fenske. Five and one-tenth square centimeters of copper per gram had approximately the same catalytic effect as 14.1 sq. cm. of iron per gram, indicating the former metal to be the more active of the two on a basis of equal areas per gram of oil. Three square centimeters per gram of lead was much less active than either copper or lead in the early stages, but became equal to them when 800 cc. of oxygen per 100 g. of oil had been absorbed. From there on it was the most active of the three metals. They found 100 p.p.m. of copper, 500 p.p.m. of lead, and 1000 p.p.m. of iron, all in the form of naphthenate, to have about the same catalytic effect. This order differs from that reported by Fenske in that the positions of lead and iron naphthenates are reversed.

In their study of the effect of surface area of metal and concentration of soluble metal, Larsen and Armfield found that curves of varying forms were obtained, depending upon the catalyst and upon the oil. They showed, for example, that three oils, a California extracted, a Mid-Continent extracted, and a Pennsylvania, could be rated in all six of the possible orders of stability at a given temperature (150°C.) and with a given catalyst (copper naphthenate), merely by altering the concentration of dissolved copper. Thus, if an oxidation test is to have significance in terms of correlation with engine behavior, the choice of catalyst, with respect to both kind and amount, must be judicious.

Larsen and Armfield studied catalysis in engines by withdrawing oil samples and determining their rate of oxidation in glass without any other added catalyst. They found that an oil loses stability very rapidly, particularly if the engine is operating without a filter. They found that a used oil could be considerably improved with respect to stability simply by filtration. Removal of fuel dilution by steam distillation had little further effect, but removal of soluble metals by washing with dilute hydrochloric acid and percolation through clay restored the oil practically to its original stability. They show that the oil insolubles, or "crankcase catalyst," constitute a potent catalyst, particularly when obtained from an engine which operated on a leaded gasoline. The copper and lead contents of crankcase catalyst are too low to account for its activity, which the authors believe is due to the presence of iron halides, derived from engine metals and the halogen compounds present in tetraethyllead fluid.

While heavy petroleum fractions have been in the past, and still are to a certain extent, used as such, the trend is toward the incorporation of additives for various specific purposes. These include the following: pour-point depressors, viscosity-index improvers, detergents, metal passivators and deactivators, and conventional antioxidants. The last-named class is, of course, of the greatest concern here, but the others cannot be ignored in oxidation studies, for they may have a profound, though indirect, effect upon rate of oxidation. Detergents, for example, peptize certain oxidation products and keep them colloidally dispersed, thus preventing their deposition as lacquer. In this manner they keep the metal surfaces of an engine clean, but vulnerable to attack by acidic oxidation products of the oil. The dissolved metals then act as catalysts for further oxidation. Passivators, on the other hand, protect metal surfaces from attack by forming a protective film on their surface, and thus prevent metal catalysis as well. Deactivators nullify the catalytic effect of dissolved metals by reacting with them and forming a non-active, or sometimes an insoluble, combination. Thus, detergents, passivators, and deactivators, none of which need have any effect upon oxidation rate in glass and in the absence of catalysts, may have a large effect in engines.

A single compound may serve in more than one capacity. Reiff (39) has shown that metal salts of phenolic acids of the following general structure have multifunctional properties. M is a metal and R an alkyl group. Compounds of this class act as pour-point depressors and viscosity-index improvers as well as inhibitors, as shown by the fact that viscosity increase, naphtha insolubles, and neutralization number are maintained at a low value in engine tests as compared to a blank run on the oil without additive.



Since the pioneer work of Moureu and Dufraisse (34), antioxidants have been investigated for stabilizing a number of products, including rubber, fats, chemicals, and gasoline as well as lubricating oil. A complete review of this field is beyond the scope of this paper, and only a few references will be cited.

The mechanism of the action of antioxidants is generally considered to be that of chain breaking, the antioxidant reacting with a "hot" molecule and thus being itself oxidized. In this process the antioxidant molecule is destroyed, but with a dissipation of the energy possessed by the "hot" molecule, so that the chain is broken. Thus the oxidation of hundreds or thousands of molecules of hydrocarbon has been prevented, since this energy is passed on from one molecule to the next in the normal chain reaction.

One of the earliest studies of antioxidants in mineral oil was reported by Haslam and Frolich (25) in 1927. They tested a number of compounds, most of which contained nitrogen, in 0.01 per cent concentration in a medicinal oil by bubbling dry oxygen through the oil at a temperature of 130°C., and followed the course of oxidation by measurements of neutralization number. The following compounds, listed in order of increasing potency, were found to be effective: diphenylguanidine, β-naphthylamine, ethyl-α-naphthylamine, *p*-aminophenol, diphenylamine, phenyl-α-naphthylamine, and unsymmetrical diphenylhydrazine.

Fenske *et al.* (19) classify antioxidants as: (*a*) hydroxy compounds (phenols, naphthols), (*b*) nitrogen compounds (amines, etc.), (*c*) sulfur compounds (disulfides, thioethers, etc.), (*d*) organometallic compounds, (*e*) halogen compounds, (*f*) compounds containing higher members of the oxygen and nitrogen groups in the Periodic Chart, such as phosphorus, arsenic, antimony, selenium, and tellurium.

Dornte (14) showed that the addition of 0.01 per cent of phenyl-α-naphthylamine to a white oil increased its induction period from practically zero to about 20 hr., after which the oxidation curve was practically parallel to the curve for the original oil. von Fuchs and Diamond (21) found that a turbine oil base stock, which approached white oils in degree of refining, responded to the addition of phenyl-α-naphthylamine in a similar fashion. On the other hand, less severely treated oils, e.g., solvent-extracted neutrals, display a far inferior susceptibility to this inhibitor. Thus the addition of antioxidant, at least of the phenyl-α-naphthylamine type, to an oil is by no means a universal "cure-all." The base stock to which it is added must be carefully refined, not for maximum stability, but for maximum inhibitor susceptibility.

Chernozhukov and Krein (8) investigated several compounds as antioxidants in their bomb test, and found catechol, hydroquinone, resorcinol, pyrogallol, quinone, aniline, and β-naphthylamine to be effective. Resins obtained from petroleum fractions by extraction with acetone were also effective. These were probably similar in character to the bright stock aromatics previously mentioned as reported by von Fuchs and Diamond (21) as increasing the stability of a motor oil when added in low concentration. Chernozhukov and Krein also report favorable preliminary results on several organic sulfur compounds, but do not list the compounds tested.

IV. CONCLUSIONS

The oxidation of a paraffin, under conditions comparable to those encountered by lubricating oils in service, tends to initiate at a beta carbon atom; that of an alkylnaphthene at a carbon in the ring to which an alkyl group is attached; and that of an alkyl aromatic at a carbon in an alkyl group adjacent to the ring. Thus, the two types of ring compounds differ in their course of oxidation in that the aromatic ring tends to remain intact, whereas the naphthene ring is ruptured at an early stage of oxidation. Branched paraffins probably oxidize at the point of branching as well as at a beta carbon. The complex molecules comprising lubricating oils probably have several carbon atoms possessing varying degrees of vulnerability. For example, a naphthene or aromatic with a long alkyl side chain would probably be oxidized at the carbon adjacent to the terminal methyl group, as governed by the rule for paraffins, as well as at the carbon in or adjacent to the ring, as influenced by the naphthene or aromatic ring, respectively.

The initial oxidation product is in every case postulated to be a hydroperoxide, which decomposes in one of three manners: by dehydration, with the liberation of a molecule of water; by reduction, with the liberation of an atom of active oxygen; or by further oxidation, with the splitting of at least one C—C bond. Aldehydes, ketones, and acids are among the principal products formed from peroxides. These can in turn undergo further oxidation or condensation. When an aromatic is oxidized, the condensation products are particularly conspicious, for they are dark in color and usually insoluble.

Paraffins and naphthenes oxidize at comparable rates. The introduction of a benzene ring at the end of a paraffin molecule causes an increase in rate, as does the introduction of olefinic unsaturation. The presence of a partially hydrogenated ring, e.g., tetralin, has a particularly great effect in increasing oxidation rate. The presence of a naphthalene nucleus, on the other hand, stabilizes the molecule to a remarkable extent. This is probably caused by an inhibitory effect by oxidation products of the naphthalene derivative (naphthols).

Mixtures of hydrocarbons do not in general oxidize as might be expected, i.e., with the least stable component reacting to the greatest extent. On the contrary, the naphthalene derivatives, which by themselves are the most stable, seem to be oxidized preferentially in mixtures. Thus, interaction effects play an important role in determining the oxidation rate of a lubricating oil.

Oxidation rate may be markedly increased by the addition of compounds of copper, lead, and iron. The effect of these catalysts is largely on the over-all rate and not upon the formation of specific products, since the distribution of oxidation products remains sensibly constant. Catalyzed oxidation may be retarded by the use of passivators or deactivators, which may both be regarded as anticatalysts in that one prevents the solution of metals, and the other reacts with soluble compounds to render them catalytically inactive. Oxidation can also be retarded by the incorporation of antioxidants, which act as chain breakers in the oxidation process.

The writer wishes to thank the Shell Oil Company for permission to publish this paper and to acknowledge the generous coöperation of the staff of the Wood River Research Laboratories in the preparation of the paper.

V. REFERENCES

(1) Baker, M. D.: Power **85,** 314 (1941).

(2) Balsbaugh, J. C., and Oncley, J. C.: Ind. Eng. Chem. **31,** 318–26 (1939).

(3) Burwell, A. W.: *Science of Petroleum,* Vol. II, pp. 1028–32. Oxford University Press, London (1938).

(4) Chavanne, G., and Bode, E.: J. Am. Chem. Soc. **52,** 1609–22 (1930).

(5) Chavanne, G., and Tock, G.: Bull. soc. chim. Belg. **41,** 630–47 (1932).

(6) Chernozhukov, N. I.: II Congr. mondial petrole, **2** Section 2, 797–802.

(7) Chernozhukov, N. I., and Krein, S. E.: J. Applied Chem. (U. S. S. R.) **10,** 435–8 (1937); Chem. Abstracts **32,** 1664 (1938).

(8) Chernozhukov, N. I., and Krein, S. E.: Neftyanoe Khoz. **23,** 242–50, 285–90 (1932); **25,** 35–8, 102–5 (1933); **28,** 59–66 (1935); Foreign Petroleum Tech. **1,** 121–34 (1933); **2,** 21–80 (1934); **3,** 577–94 (1935); **4,** 13–32 (1936).

(9) Clark, F. M.: Ind. Eng. Chem. **31,** 327–33 (1939).

(10) Clark, F. M., and Raab, E. C.: Ind. Eng. Chem. **34,** 110–16 (1942).

(11) Danilovich, A., and Dianina, T.: Masloboĭno Zhirovoe Delo **1935,** 15–18; Chem. Abstracts **29,** 5255 (1935).

(12) Davis, L. L., Lincoln, B. H., Byrkit, G. D., and Jones, W. A.: Ind. Eng. Chem. **33,** 339–50 (1941).

(13) Denison, G. H.: Ind. Eng. Chem. **36,** 477–82 (1944).

(14) Dornte, R. W.: Ind. Eng. Chem. **28,** 26–30 (1936).

(15) Dornte, R. W. and Ferguson, C. V.: Ind. Eng. Chem. **28,** 863–6 (1936).

(16) Dornte, R. W., Ferguson, C. V., and Haskins, C. P.: Ind. Eng. Chem. **28,** 1342–5 (1936).

(17) Dupont, P., and Chavanne, G.: Bull. soc. chim. Belg. **42,** 537–43 (1933).

(18) Evans, E. A.: World Petroleum Congr., London, 1933, Proc. **2,** 460–1.

(19) Fenske, M. R., Stevenson, C. E., Lawson, N. D., Herbolsheimer, G., and Koch, E. F.: Ind. Eng. Chem. **33,** 516–24 (1941).

(20) Fenske, M. R., Stevenson, C. E., Rusk, R. A., Lawson, N. D., Cannon, M. R., and Koch, E. F.: Ind. Eng. Chem., Anal. Ed. **13,** 51–60 (1941).

(21) Fuchs, G. H. von, and Diamond, H.: Ind. Eng. Chem. **34,** 927–37 (1942).

(22) George, P., Rideal, E. K., and Robertson, A.: Nature **149,** 601–2 (1942).

(23) Goswami, M., and Chatterjee, S. K.: J. Indian Chem. Soc. **8,** 533 (1931); Chem. Abstracts **26,** 693 (1932).

(24) Hanson, T. K., and Egerton, A. C.: Inst. Mech. Engrs., Lubrication Discussion, October, 1937, Group IV, 122–7; Chem. Abstracts **32,** 6040 (1938).

(25) Haslam, R. T., and Frolich, P. K.: Ind. Eng. Chem. **19,** 292–6 (1927).

(26) Hicks-Bruun, M. M., Ritz, B. L., Ledley, R. E., and Bruun, J. H.: Ind. Eng. Chem. **36,** 562–8 (1944).

(27) Hock, H., and Lang, S.: Ber. **75B,** 300–13, 1051–4 (1942).

(28) King, E. O.: Proc. Roy. Soc. (London) **A139,** 447–59 (1933); J. Inst. Petroleum Tech. **20,** 97 (1934).

(29) Lamb, G. G., Loane, C. M., and Gaynor, J. W.: Ind. Eng. Chem., Anal. Ed. **13,** 317–21 (1941).

(30) Larsen, R. G., and Armfield, F. A.: Ind. Eng. Chem. **35,** 581–8 (1943).

(31) Larsen, R. G., and coworkers, Shell Development Company: Unpublished work.

(32) Larsen, R. G., Thorpe, R. E., and Armfield, F. A.: Ind. Eng. Chem. **34,** 183–93 (1942).

(33) Likhushin, K. P., Masumyan, V., and Levkupulo, N.: Azerbaĭdzhanskoe Neftyanoe Khoz. **1934,** No. 10, 77–82, No. 11–12, 87–9; Chem. Abstracts **29,** 6034, 7629 (1935).

(34) Moureu, C., and Dufraisse, C.: Compt. rend. **175,** 127–329 (1922); **176,** 624–9 (1923); Compt. rend. soc. biol. **86,** 321–3 (1922); British patent 181,365 (1922).

(35) Petrov, G. S.: Trans. VI Mendeleev Congr. Theoret. Applied Chem. 1932, **2**, Pt. 1, 844–67 (1935); Chem. Abstracts **30**, 3975 (1936).

(36) Plisov, A. K.: Bull. soc. chim. [5] **3**, 1274–81 (1936); Chem. Abstracts **30**, 7829 (1936).

(37) Plisov, A. K., Galendeev, V. P., and Zel'tsburg, A. I.: J. Applied Chem. (U. S. S. R.) **9**, 844–5 (1936); Foreign Petroleum Tech. **4**, 237–43 (1936).

(38) Plisov, A. K., and Grebennikova, M. D.: J. Gen. Chem. (U. S. S. R.) **9**, 547–56 (1939); Chem. Abstracts **33**, 9603 (1939).

(39) Reiff, O. M.: Ind. Eng. Chem. **33** 351–7 (1941).

(40) Shoruigin, P. P., and Kreshkov, A. P.: J. Gen. Chem. (U. S. S. R.) **3**, 825–30 (1933); **4**, 988–94 (1934); Chem. Abstracts **28**, 6106 (1934); **29**, 2147 (1935).

(41) Stevens, H. N.: J. Am. Chem. Soc. **48**, 1824–6, 2920–1 (1926); **50**, 2523–9 (1928).

(42) Stevens, H. N., and Roduta, F. L.: J. Am. Chem. Soc. **57**, 2380–1 (1935).

(43) Varlamov, V.: Maslaboĭno Shirovoe Delo **1932**, No. 4–5, 41–5; No. 6, 47–53; Chem. Abstracts **27**, 3595, 5955 (1933).

(44) Velikovskiĭ, D. S., and Lemer, A. P.: Neftyanoe Khoz. **1937**, No. 2, 63–7; Chem. Abstracts **32**, 4764 (1938).

(45) Velikovskiĭ, D. S., and Vasil'eva, O. V.: Neftyanoe Khoz. **29**, No. 9, 62–7 (1935); Foreign Petroleum Tech. **4**, 209–35 (1935).

THE SYNTHESIS OF AROMATIC ALDEHYDES

LLOYD N. FERGUSON[1]

Department of Chemistry, University of California, Berkeley, California

Received August 5, 1944
Revised copy received November 21, 1945

CONTENTS

I. Introduction 228
II. Direct methods 228
  A(1). Gattermann and Koch reaction 228
  A(2). Gattermann reaction 229
  B. Reimer–Tiemann reaction 229
  C. Duff's hexamethylenetetramine reaction 230
  D. Reactions with *N*-methylformanilide 230
  E. Formaldehyde condensations 231
  F. Chloral condensations 232
  G. Condensations with alloxan, mesoxalates, oxalyl ester acid chlorides, and sodium glyoxylate 232
    (1) Alloxan 232
    (2) 1,2-Diketo esters and mesoxalates 233
    (3) Oxalyl ester acid chlorides 233
    (4) Sodium glyoxylate 234
  H. Mercury fulminate condensations 234
  I. Chloromethylene dibenzoate condensations 234
  J. Isatin chloride condensations 234
  K. Reactions with organolithium compounds 235
  L. Naphthaldehydes from benzofurans and acrolein 235
III. Indirect methods 235
  A. Oxidation of unsaturated side chains 235
  B. Oxidation of benzyl alcohols 238
  C. Oxidation of benzyl halides 239
  D. Oxidation of benzylanilines, benzylamines, *N*-alkylanilines, phenylnitromethanes, and phenylacetic acids 239
  E. Oxidation of olefinic side chains 239
  F. Oxidation of acetophenones 240
  G. Oxidation of naphthalenes 241
  H. Reduction of acids 241
  I. Reduction of esters 242
  J. Reduction of acid chlorides 243
  K. Reduction of nitriles 243
  L. Reduction of amides and imidochlorides 244
  M. Reduction of hydrazides 244
  N. Aldehydes from Grignard reagents 245
  O. Aldehydes from benzophenone oxime 246
IV. Non-preparative reactions 248
V. Yields in the synthesis of aldehydes 248
VI. References 248

---

[1] Present address: Department of Chemistry, Howard University, Washington, D. C.

I. INTRODUCTION

This review is a survey of the literature for methods used to synthesize aromatic aldehydes and will be limited to a discussion of those compounds in which the formyl group is directly attached to the aromatic nucleus. Processes by which aldehydes are obtained from natural sources or by fermentation will not be considered.

Examination of the literature reveals the fact that most investigations have been carried out on benzene and its substitution products. In only a few instances have the reactions been extended to other aromatic nuclei. Unfortunately, per cent yields or limitations for some of the methods have not been reported.

There are two general routes for the synthesis of aromatic aldehydes (110): (*1*) *Direct methods.* These procedures involve the direct introduction of the formyl group, or groups directly changeable into it, into the aromatic nucleus. (*2*) *Indirect methods.* By this term is meant the conversion of groups already in the nucleus into aldehyde groups.

In choosing any one of the methods to produce an aromatic aldehyde it is important to consider the effect of groups already in the nucleus, since these influence orientation, activation, or deactivation of the ring and side reactions with the reagents.

II. DIRECT METHODS

A(1). Gattermann and Koch reaction

$$C_6H_6 + CO + HCl \xrightarrow{AlCl_3} C_6H_5CHO$$ [2]

Gattermann and Koch (75) first reported the method of preparing aromatic aldehydes by reacting benzene (and its substitution products) with carbon monoxide and hydrogen chloride, using anhydrous aluminum chloride as a catalyst.

The two gases, carbon monoxide and hydrogen chloride, are bubbled for a suitable period through an ether or nitrobenzene solution containing the aromatic compound, cuprous chloride, and aluminum chloride. Ice is then added and the aldehyde steam-distilled from the reaction mixture (171). If the reaction is run under elevated pressures of carbon monoxide, the hydrogen chloride and the aluminum chloride activators (chlorides of copper, iron, or nickel) are not required (102, 123). In this case, to shorten the induction period, a trace of water or hydrogen chloride is added, or better, a residue of aldehyde–aluminum chloride complex is left in the reaction chamber (127). Nickel and iron carbonyls have been used as sources of carbon monoxide, but the resulting yields of aldehydes were less than 40 per cent (98, 293).

Under normal pressures the yields average only 30–50 per cent, but with high carbon monoxide pressures they run 80–90 per cent. The reaction is not applicable to phenols or their alkyl ethers. Only monosubstitution takes place, usually in the para-position (171, 201). If the para-position is occupied, migration from this position is known to occur (63). Thus, *p*-xylene will give 2,4-dimethylbenzaldehyde.

---

[2] The equations used throughout this paper are not balanced, but merely indicate reactants and important products.

A(2). Gattermann reaction

$$C_6H_6 + HCN + HCl \xrightarrow{AlCl_3} C_6H_5CH{=}NH{\cdot}HCl \xrightarrow{H_2O} C_6H_5CHO$$

In 1906 Gattermann altered the Gattermann-Koch reaction by using dry hydrogen cyanide in place of carbon monoxide (70). This modification produces the aldimine hydrochloride as an intermediate which must then be hydrolyzed to the aldehyde.

The Gattermann reaction, unlike the Gattermann and Koch reaction, can be applied to phenols and aromatic ethers. With only one hydroxyl or alkoxyl group in the ring, anhydrous aluminum chloride is recommended for the catalyst, but meta-substituted di- and tri-hydric phenols, or their alkyl ethers, react when a milder catalyst such as anhydrous zinc chloride is used, and a few will react even without a catalyst (71, 72, 73, 75, 242). Monohydric phenols will react with hydrogen cyanide to yield aryl formididate hydrochlorides, $C_6H_5OCH{=}NH{\cdot}HCl$, but without a catalyst rearrangement to the aldimine will not occur (105). The Gattermann reaction has been extended to mono- and di-hydroxynaphthalenes.

Adams (1, 2) introduced a more convenient method for carrying out this reaction by using zinc cyanide in place of hydrogen cyanide. Pure zinc cyanide is ineffective. However, with a trace of added potassium or sodium chloride zinc cyanide works very satisfactorily (8).

B. Reimer–Tiemann reaction

$$C_6H_5OH + CHCl_3 + NaOH \rightarrow HOC_6H_4CHO$$

The production of aromatic hydroxy aldehydes from phenols, chloroform, and aqueous alkali was described by Reimer and Tiemann in 1876 (216).

The phenol, chloroform, and aqueous alkali are refluxed at a moderate temperture for several hours. Then the reddish-violet reaction mixture is acidified, and the aldehyde isolated by steam distillation or crystallization (7, 225). A milder base such as sodium carbonate is sometimes used in place of the sodium hydroxide (101). If pyridine is substituted for the aqueous alkali, only the ortho-isomer is obtained (241, 271). Bromoform, iodoform, or trichloroacetic acid will serve equally as well as the chloroform but are more expensive. Side-products containing chlorine or ketone groups are known to be produced (7, 10, 133, 271).

The ortho:para ratio is usually less than unity (101) and occasionally traces of dialdehydes are recovered. Yields seldom run over 50 per cent and are less than 25 per cent when the ring contains such negative groups as sulfo, nitrile, carboxyl, or nitro, which deactivate the nucleus (271).

Chaudhuri (31) has found that chloroform, potassium hydroxide, and benzaldehyde will react to give *o*- and *m*-dichloromethylbenzaldehydes, which can be hydrolyzed to the corresponding phthalaldehydes. Rapson, Saunder, and Stewart (215) were unable to repeat Chaudhuri's work.

C. *Duff's hexamethylenetetramine reaction*

$$C_6H_5OH + (CH_2)_6N_4 \rightarrow HOC_6H_4CH{=}NCH_3 \rightarrow HOC_6H_4CHO$$

Hexamethylenetetramine will condense with phenols to form intermediate products which, when hydrolyzed in mineral acids, will give small amounts of aromatic hydroxy aldehydes (50). Duff has worked out a general procedure resulting in slightly improved yields (48).

The phenol is heated at elevated temperatures for 10–30 min. in a mixture of hexamethylenetetramine, boric acid, and glycerol. The resulting dark brown viscous liquid is acidified with dilute sulfuric acid, and the aldehyde isolated by steam distillation.

Only ortho substitution, or a small amount of ortho–para disubstitution, takes place. The yields, averaging only 15–20 per cent, are lower than those from the Reimer–Tiemann reaction, but this method offers the advantage of producing a much purer product and requires less time. Negative groups hinder or prevent the reaction from taking place, while resorcinol and phloroglucinol rapidly form resins under the conditions of the reaction (271). This method does not apply for hydroxypyridines and hydroxyquinolines (131, 271).

Duff (49) found that the above procedure does not succeed with *N*,*N*-dialkylanilines, but that with a slight modification satisfactory results may be obtained. In place of the glyceroboric acid, which was found unsuitable, a mixture of formic acid and acetic acid is used. Either acid alone is unsatisfactory. Duff believes that the formic acid has reducing action, as indicated by the evolution of carbon dioxide during the reaction. The yields of *p*-*N*,*N*-dialkylamino- or *p*-*N*,*N*-alkylarylamino-benzaldehydes average 35–45 per cent.

D. *Reactions with N-methylformanilide*

$$C_6H_5NR_2 + HCON(CH_3)C_2H_5 \xrightarrow{POCl_3} R_2NC_6H_4CHO + C_6H_5NHCH_3$$

Vilsmeier and Haack (273) found *N*,*N*-dialkylanilines and *N*-methylformanilide to condense in the presence of phosphorus oxychloride, producing intermediates which, when hydrolyzed in acid solution, will give *p*-(*N*,*N*-dialkylamino)benzaldehydes in 80 per cent yields. *N*-Methylformanilide alone, when reacted with phosphorus oxychloride and phosphorus pentachloride, will give a 37 per cent yield of *p*-(*N*-methylamino)benzaldehyde.

As a result of their work an excellent method for the preparation of aldehydes has been developed, but it is limited in its applicability (57, 188, 207). Benzene and naphthalene will not react; however, their alkoxy or *N*,*N*-dimethylamino derivatives, naphthols, and certain hydrocarbons which contain active hydrogen atoms will give yields averaging 70–85 per cent.

The aromatic derivative, *N*-methylformanilide, and phosphorus oxychloride are allowed to react at a suitable temperature; then an aqueous solution of sodium acetate is added and the mixture steam-distilled to liberate the aldehyde. Table 1 will serve to illustrate the behavior of several compounds in this reaction (56, 58, 271, 274).

Compounds other than phosphorus oxychloride which will serve as catalysts are halogen acid anhydrides, such as thionyl chloride, oxalyl chloride, or phosgene (271). In the presence of aluminum chloride or the above oxychlorides of phosphorus and sulfur, *N*, *N*-dimethylaniline will react with a large excess of formamide to give *p*-(*N*, *N*-dimethylamino)benzaldehyde (113, 114, 177).

TABLE 1

Reaction with N-methylformanilide

COMPOUND	POSITION OF SUBSTITUTION	YIELD
		per cent
Anthracene	9	80–92
Acenaphthene	3	85
β-Ethoxynaphthalene	1	74–85
o-Chloroanisole	?	3
Resorcinol dimethyl ether	2	95
N,*N*-Diethylaniline	4	80
1,2-Benzanthracene	10	64
Pyrene	3	53
1,2,5,6-Benzanthracene		No reaction
3,4-Benzpyrene	5	90
Hydrindene		No reaction
α-Methylnaphthalene		No reaction
Phenanthrene		No reaction
Crysene		No reaction

E. Formaldehyde condensations

$$\begin{tikzcd}
C_6H_5OH + HCHO \arrow[r] & HOC_6H_4CH_2OH \arrow[r] \arrow[dr, "C_6H_5NO \text{ or } C_6H_5NHOH"] & HOC_6H_4CHO \\
 & & HOC_6H_4CH{=}NC_6H_5 \arrow[u]
\end{tikzcd}$$

Anilines and phenols condense with formaldehyde in dilute acid or base to give substituted derivatives of benzyl alcohol, which may be isolated or oxidized in the same reaction mixture to the corresponding aldehydes (47, 78, 96, 178, 238, 268, 278). This method has been used largely for the technical preparation of amino- and hydroxy-benzaldehydes.

The aniline or phenol, dilute formaldehyde, aqueous acid or alkali, and the oxidant are mixed, allowed to react for several hours at a suitable temperature, acidified, and the aldehyde isolated by steam distillation (175). Nitrosobenzene or phenylhydroxylamine is used for the oxidizing agent and cupric oxide as a catalyst.

Para substitution predominates, with yields running as high as 85 per cent but averaging only 40–50 per cent. Some processes use iron, aluminum, or electrolytic reduction to reduce nitro- and nitroso-benzenes to phenylhydroxylamines, and the reduction is carried out simultaneously with the formaldehyde condensation (118, 239, 275). This reduction–condensation technique gives lower yields than the straight condensation method.

F. Chloral condensations

$$C_6H_5OH + CCl_3CHO \rightarrow HOC_6H_4CHOHCCl_3 \rightarrow HOC_6H_4CHO$$

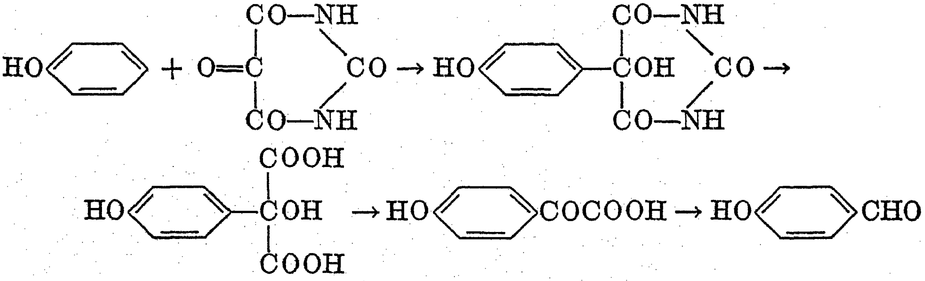
Phenols condense with chloral hydrate in dilute base to give, along with small amounts of further condensation products, hydroxyphenyltrichloromethylcarbinols which can be oxidized to the corresponding hydroxybenzaldehydes (185, 190, 199, 209). The phenols and chloral hydrate are kept alkaline with potassium carbonate for six to twelve weeks, whereupon the carbinols are produced in 60–80 per cent yields. The latter are either treated with zinc dust in aqueous alcohol and the product oxidized with chromium trioxide, or oxidized directly to the aldehydes with dilute alkaline sodium dichromate. The hydroxybenzaldehydes are obtained in 40–60 per cent yields on the basis of the amount of phenol used.

Substitution takes place in the para-position unless it is occupied and then ortho substitution occurs, but to a lesser extent.

G. Condensation with alloxan, mesoxalates, oxalyl ester acid chlorides, and sodium glyoxylate

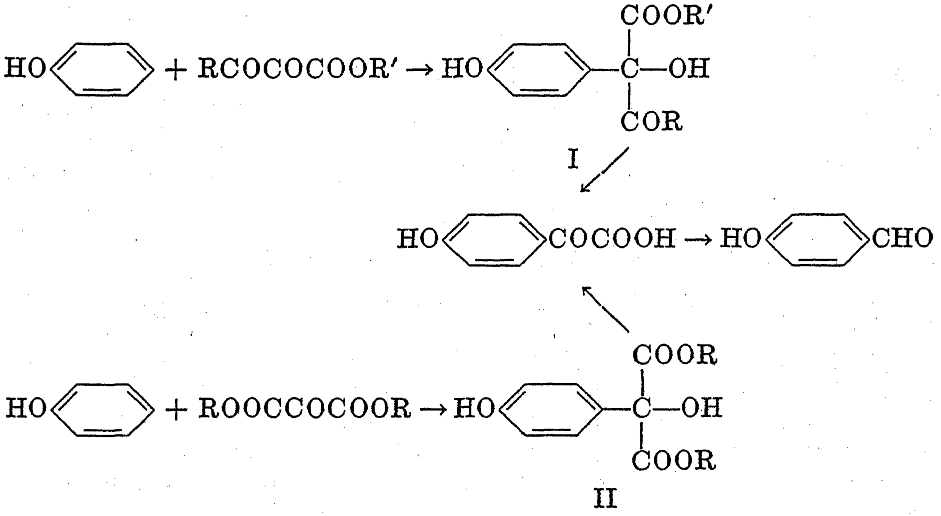
Alloxan, mesoxalates, oxalyl ester acid chlorides, 1,2-diketo esters, and sodium glyoxylate condense with phenols to produce intermediates which can be converted into phenylglyoxylic acids, and the latter can be decarboxylated to the corresponding benzaldehydes.

(1) Alloxan



Phenols and alloxan, in the presence of hydrogen chloride, concentrated sulfuric acid, or anhydrous zinc chloride, condense similarly to an aldol condensation (22, 238). The products are hydrolyzed in dilute alkali and the hydroxymalonic acid derivatives, which are freed by acidification, are heated in acid solution, whereupon they lose carbon monoxide and water to produce the phenylglyoxylic acids. These are decarboxylated in boiling *p*-toluidine to produce the corresponding hydroxybenzaldehydes.

(2) 1,2-Diketo esters and mesoxalates



Phenols and anilines condense with 1,2-diketo esters or mesoxalates in acetic acid, anhydrous zinc chloride being used as catalyst, to give aldol-type condensation products, I and II, in 75–85 per cent yields (86). To obtain the phenylglyoxylic acids, compounds of type I are refluxed with an aqueous solution of cupric acetate, while those of type II are first hydrolyzed in dilute potassium hydroxide, neutralized, and then refluxed with aqueous cupric sulfate. The phenylglyoxylic acids give 68 per cent yields of the corresponding aldehydes when heated with *N*, *N*-dimethylaniline and up to 97 per cent yields when heated at 170°C. in *p*-(*N*, *N*-dimethyl)toluidine.

Diketosuccinic acid, HOOCCOCOCOOH, when substituted for its diester in the condensation with phenol, gives only a 15 per cent yield of the *p*-hydroxyphenylglyoxylic acid.

(3) Oxalyl ester acid chlorides

$$C_6H_6 + COORCOCl \rightarrow C_6H_5COCOOR \rightarrow C_6H_5CHO$$

Bouveault discovered that ethyl oxalate acid chloride condenses with benzene and its substitution products in the presence of anhydrous aluminum chloride to give the esters of phenylglyoxylic acids in yields, usually, above 70 per cent (24). The phenylglyoxylates have also been obtained in small yields from diphenylmercury and ethyl oxalate acid chloride. Besides the methods given above for the decarboxylation of the phenylglyoxylic acids, Bouveault frequently prepared the hydrazine, hydroxylamine, or aniline Schiff's base of the phenylglyoxylic acid and then heated this to bring about decarboxylation to the corresponding aldehyde Schiff's base. Derivatives of toluene and anisole are especially suitable for this method of preparing the corresponding aldehydes.

(4) Sodium glyoxylate

$$C_6H_5OH + CHOCOONa \rightarrow HOC_6H_4CHOHCOONa \rightarrow HOC_6H_4CHO$$

Monoalkyl ethers of catechol will condense with sodium glyoxylate, and the products may be converted into the corresponding substituted benzaldehydes by refluxing with aqueous cupric sulfate or nitrobenzene (32, 179, 186, 220). Yields up to 80 per cent are obtained, but the method has only been extended to a few derivatives of catechol.

H. Mercury fulminate condensations

$$C_6H_6 + Hg(ONC)_2 \xrightarrow{AlCl_3} C_6H_5CH{=}NOH \rightarrow C_6H_5CHO$$

Benzene and its alkyl derivatives react with mercury fulminate in the presence of aluminum chloride to give moderate yields of oximes which may then be hydrolyzed to the corresponding aldehydes (235, 236). The reaction is run at 45°C. (mercury fulminate explodes when heated to about 180°C.). If a solvent is necessary it is usually nitrobenzene; carbon disulfide and ligroin are unsatisfactory.

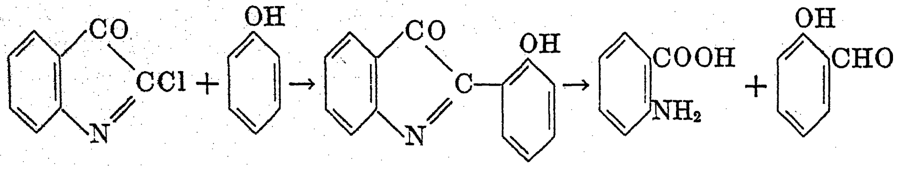
Scholl discovered that if sublimed aluminum chloride is used as the catalyst substituted benzonitriles are the main products, while traces of substituted benzaldehydes and benzamides are formed as side products. But, by using a mixture of sublimed aluminum chloride, crystallized aluminum chloride hexahydrate, and dried aluminum hydroxide, a 65–70 per cent yield of the oxime is obtained. Polyhydric phenols give excellent yields, phloroglucinol giving almost a quantitative yield. Ortho and para substitution are both found to take place.

I. Chloromethylene dibenzoate condensations

$$(C_6H_5COO)_2CHCl + C_6H_5OR \xrightarrow{AlCl_3} (C_6H_5COO)_2CHC_6H_4OR \rightarrow ROC_6H_4CHO$$

Wenzel found that when chloromethylene dibenzoate and anhydrous aluminum chloride are added to a benzene solution of mesitylene an intermediate is formed which, upon hydrolysis in hydrochloric acid, produces mesitylaldehyde (285). Likewise, anisole reacts with chloromethylene dibenzoate to give an 80 per cent yield of anisaldehyde, while benzene only produces a diphenylcarbinol benzoate.

J. Isatin chloride condensations



Upon refluxing in benzene solution, phenols and naphthols condense with isatin chloride to give the corresponding 2-indolindigo derivatives which may be hydrolyzed in 10 per cent sodium hydroxide to produce anthranilic acid and the *o*-hydroxybenzaldehyde or naphthaldehyde in 20–40 per cent over-all yields (18, 65, 231).

K. Reactions with organolithium compounds

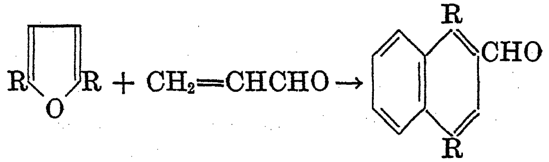
$$C_6H_5OR + R'Li \rightarrow ROC_6H_4Li \xrightarrow{HC(OR)_3} ROC_6H_4CH(OR)_2 \rightarrow ROC_6H_4CHO$$

When phenyllithium derivatives are heated with *N*-methylformanilide or ethyl orthoformate, intermediates are formed which, upon acid hydrolysis, produce substituted benzaldehydes in 70 per cent yields (270, 291, 292).

The hydrogen atoms which are ortho to an alkoxyl group in the benzene nucleus are sufficiently active to be readily replaced by lithium. A convenient method is to effect an exchange with phenyl- or butyl-lithium. Thus anisole, after several hours heating at 100°C. with phenyllithium, gives a 70 per cent yield of 2-methoxyphenyllithium (291, 292).

Should the phenyllithium derivative be prepared through an exchange of lithium for a halogen atom in an aromatic ring, the method would fall into the "indirect method" classification and would then parallel the Grignard method, for which magnesium is used.

L. Naphthaldehydes from benzofurans and acrolein



Substituted benzofurans have been reacted with acrolein by refluxing in ethyl alcohol; after addition of hydrochloric acid, substituted naphthaldehydes were isolated in 50 per cent yields (283, 284).

III. INDIRECT METHODS

As stated above, the processes whereby groups on the aromatic nucleus are converted into aldehyde groups have been classified as indirect methods. Most of these consist of oxidation or reduction of side chains to the formyl group. Thus, the —CH_2Y group, where Y represents a hydrogen, hydroxyl, halogen, nitro, or carboxyl group, may be oxidized and the COOH, COCl, COOR, CN, $CONR_2$, and $CHNNR_2$ groups may each be reduced to the aldehyde group.

A. Oxidation of saturated side chains

$$C_6H_5R + \text{oxidant} \rightarrow C_6H_5CHO$$

(*1*) *Electrolytic oxidation:* There have been many attempts to oxidize side-chain alkyl groups to aldehyde groups, but few have met with success (157).

The products from the electrolysis of toluene, as reported by several authors, are chiefly ethyl benzoate, benzoic acid, *p*-sulfobenzoic acid, quinone, hydroquinone, and benzaldehyde, depending upon the experimental conditions (54, 109, 151, 218).

Following their experiments with toluene emulsified in sulfuric acid, Fichter and Stocker proposed that the ring is attacked more rapidly than the side chain, giving rise to polyhydroxybenzenes and subsequent decomposition (55). Mann and Paulson, using platinum sheet electrodes (140), attempted to determine the conditions for a maximum yield of benzaldehyde from toluene regardless of side products. With yields below 19 per cent their results indicate that a diaphragm around the anode is necessary and that solvents such as acetone, alcohol, or chloroform are unsatisfactory. Kawada investigated the conditions for a maximum yield of benzaldehyde from toluene, using manganous sulfate in sulfuric acid for an electrolyte and lead-plate electrodes; he concluded that a diaphragm and vigorous stirring are necessary (115). Law and Perkin obtained aldehydes from alkylbenzenes in yields up to 35 per cent, using platinum electrodes and acetone–sulfuric acid solutions for an electrolyte (128). Ethylbenzene gave mainly methylphenylcarbinol.

(*2*) *Chemical oxidation:* Most investigations of the vapor-phase oxidation of toluene have been directed toward the technical production of benzaldehyde (108, 124, 125, 141, 143, 180, 202). Air, diluted usually with nitrogen to prevent total destruction of the hydrocarbon, is most frequently used as the oxidant, while occasionally substances such as methanol, which are more readily oxidized than the toluene, are added to serve as protective agents. Experiments have largely made use of recirculating continuous processes with varied contact periods, temperature ranges up to 550°C., and catalyst carriers of asbestos, pumice, zeolites, talc, or porcelain. The relative proportions of benzyl alcohol, benzaldehyde, and benzoic acid produced are determined by the conditions. In general, oxides of molybdenum, tungsten, zirconium, and tantalum catalyze oxidations to benzaldehyde and are not active to promote further oxidation to any great extent. Oxides of manganese, copper, nickel, chromium, and uranium are more powerful and favor formation of aldehydes, but they also promote further oxidation to complete combustion of the toluene, while oxides of cobalt and cerium lead to complete combustion. Oxides of titanium, bismuth, and tin are only slightly active and require such high temperatures to initiate reaction that the toluene ignites. Vanadium pentoxide is particularly active in the oxidation of aldehydes to acids (146). Certain salts catalyze the oxidation of benzaldehyde to benzoic acid at a faster rate than the oxidation of toluene to benzaldehyde and naturally only a small yield of the aldehyde can be expected.

Oxidation of side chains consisting of straight-chain alkyl groups containing two or more carbon atoms produces chiefly ketones, since it is the carbon atom adjacent to the ring which is most readily attacked.

The heterogeneous liquid-phase oxidation of substituted toluene derivatives to the corresponding benzaldehydes has been very successful. One of the important technical methods of producing benzaldehyde consists in the treatment of toluene with manganese dioxide in 65 per cent sulfuric acid at 40°C. Salicylaldehyde also is produced on a large scale from *o*-cresol by this method, the hydroxyl group being protected from oxidation by ester formation with sulfonic or carbonic acids (6, 64, 191, 245). The oxides of lead, nickel, cobalt, cerium, selenium, and tellurium and ammonium persulfate have all been used more or less successfully as oxidants (129, 192).

A good laboratory method for oxidizing methyl groups to aldehyde groups in 40–50 per cent yields is the use of chromium trioxide in acetic anhydride (170). The diacetates are formed, preventing further oxidation, and crystallize out as sharp-melting products which may then easily be hydrolyzed in acid solution to the corresponding aldehydes (193, 260). Xylenes will give phthalaldehydes by this method.

The Étard reaction makes use of chromyl chloride, an oxidant which is reported to have just the potential required to oxidize a methyl group to a formyl group (52, 53). The chromyl chloride is added slowly to a carbon disulfide or carbon tetrachloride solution of the substituted toluene derivative, from which a dark brown, insoluble, explosive intermediate is precipitated. This is decomposed in dilute sulfurous acid to give the aldehyde. Yields up to 80 per cent are sometimes obtained. Alkyl groups other than the methyl group lead to various products. Thus, ethylbenzene gives phenylacetaldehyde; propylbenzene gives benzaldehyde, benzyl methyl ketone, and chlorinated products; and cymol gives cuminaldehyde plus an unidentified ketone (23, 129, 156, 208, 219, 220).

The methyl group of substituted toluenes may be dichlorinated or dibrominated and the benzal chloride or bromide hydrolyzed to the corresponding aldehyde (253). Large amounts of benzaldehyde are produced technically by illuminated chlorination of toluene until a certain density is reached, treatment of the mixture of mono-, di-, and tri-chlorotoluenes with aqueous calcium hydroxide or carbonate, and isolation of the aldehyde by steam distillation. Small amounts of benzoic acid are recovered from the distilling liquid after cooling (64, 139, 172). In the case of cresols, the carbonate or acetate esters are first prepared in order to prevent nuclear halogenation (38, 240, 271).

By treating nitrotoluenes with either a solution of alcoholic-aqueous sodium sulfide, a solution of sulfur in concentrated alcoholic-aqueous sodium hydroxide, or fuming sulfuric acid, oxidation and reduction within the same molecule will occur and produce aminobenzaldehydes and aminonaphthaldehydes in yields up to 70 per cent (16, 101, 194, 263). Occasionally aldehydes are not produced and sometimes toluidines and aminobenzoic acids are formed as the main products; hence the method is not general (271).

Methyl groups which are activated by ortho- or para-situated nitro groups are smoothly condensed with nitrosobenzenes, and the resulting Schiff's bases are hydrolyzed to the corresponding aldehydes in over-all yields up to 88 per cent (158, 173, 195, 228).

o-Nitrotoluene reacts with mercuric oxide in dilute sodium hydroxide to produce a mercury salt which may be oxidized directly to *o*-nitrobenzaldehyde, or halogenated to give *o*-nitrobenzal halide and the latter hydrolyzed to liberate *o*-nitrobenzaldehyde. The over-all yields of *o*-nitrobenzaldehyde are reported to be as high as 84 per cent (216a).

Toluene derivatives react with alkyl nitrites in alcoholic solutions of sodium alkoxide to convert the methyl group to an aldehyde group in 50–70 per cent yields (5, 137, 261). In the case of nitrotoluenes, the corresponding oxime is isolated and must then be hydrolyzed to the aldehyde.

Dibenzyl, prepared from benzene and 1,2-dichloroethane using anhydrous aluminum chloride as catalyst, has been oxidized to benzaldehyde by molecular oxygen at 150–280°C. (40, 234).

The oxidation of β-(5-chloro-6-methoxy-2-naphthoyl)propionic acid with alkaline sodium hypochlorite gives a 39 per cent yield of 5-chloro-6-methoxy-2-naphthaldehyde (120).

Phenylbromoacetamides, upon treatment with sodium hydroxide, will give the corresponding derivatives of benzaldehyde. The procedure has not been worked out for the general preparation of aromatic aldehydes (169).

B. Oxidation of benzyl alcohols

$$C_6H_5CH_2OH + \text{oxidant} \rightarrow C_6H_5CHO$$

A clean-cut laboratory method for oxidizing hydroxymethyl groups to aldehyde groups in yields up to 90 per cent is through the use of chromium trioxide in acetic acid or anhydride (250).

A cheaper method and one that is used more often as a technical process makes use of sulfonic or carbon acids of *m*-nitrobenzene in basic media (89, 181, 203, 270, 278). This method has shown more applicability when there are also hydroxyl groups attached to the nucleus of the benzyl alcohol since, through solution in the aqueous alkali, there results a homogeneous reaction mixture.

Dichromates in dilute sulfuric acid have been used to oxidize hydroxymethyl to formyl groups in 80–95 per cent yields (134, 300). Alkaline sodium hypochlorite has been used as an oxidant, with simultaneous steam distillation of the aldehyde as it is produced (204). Selenium dioxide (9), tellurium dioxide (62), and chloroform solutions of nitrogen trioxide or tetroxide (34) will oxidize benzyl alcohol to benzaldehyde in yields of 40 per cent or less.

A schematic method for converting a substituted benzyl alcohol into the corresponding benzaldehyde is to reflux the alcohol with a less volatile aldehyde, using an aluminum alkoxide catalyst. The equilibrium which is set up is displaced by removing the resulting less volatile aldehyde as it is formed. Thus, benzaldehyde was prepared from benzyl alcohol and cinnamaldehyde in 95 per cent yield, while the cinnamyl alcohol was recovered in 87 per cent yield (43). Anisaldehyde has also been used to prepare benzaldehyde from benzyl alcohol (227).

The vapor-phase oxidation of benzyl alcohol by air or oxygen under reduced pressures has been conducted with yields up to 90 per cent by using such catalysts as silver metal, cupric oxide, or vanadium pentoxide at temperatures of 100–350°C. (4, 44a, 163, 206, 245).

C. Oxidation of benzyl halides

$$C_6H_5CH_2X + \text{oxidant} \rightarrow C_6H_5CHO$$

A simple reaction for converting substituted benzyl halides to the corresponding benzaldehyde derivatives, and one that is quite general, is Sommelet's reaction, which consists in refluxing the benzyl halide with hexamethylenetetramine in dilute alcohol, acidifying, and isolating the aldehyde by steam distillation (36, 196, 254, 271). The yields average 60–70 per cent.

Nitrates of copper, lead, or calcium in dilute nitric acid have often been used to oxidize benzyl halides to the corresponding benzaldehydes, usually resulting in low yields (60, 214, 237, 246, 271). Oxidation with alkaline dichromates will sometimes give yields up to 90 per cent (20, 134). The refluxing of selenium dioxide with substituted benzyl chlorides produces a 45–55 per cent yield of the substituted benzaldehyde (61).

Vapor-phase oxidation of benzyl chlorides to the corresponding benzaldehydes has been carried out to give 70–90 per cent yields (245, 248).

D. Oxidation of benzylanilines, benzylamines, N-alkylanilines, phenylnitromethanes, and phenylacetic acids

The oxidation of benzylanilines in acetone solution with dichromates or permanganates produces benzalanilines, which may be hydrolyzed in mineral acid to liberate the substituted benzaldehydes (35, 197, 228).

In alkaline media, hydroxybenzylamines have been oxidized by air to hydroxybenzaldehydes, using isatin or potassium isatin-5-sulfonate as a catalyst (182, 187).

Prolonged oxidation of tribenzylamines with such oxidants as aqueous bromine, chromic acid, or thionyl chloride has been found to produce benzaldehydes (37, 132, 149, 153). Various substituted benzaldehydes and naphthaldehydes have been prepared when an arylalkylamine was heated with a chlorobenzene containing an active chlorine atom and the intermediate product oxidized in acid solution (183). The chlorinated reagents used were 2,4-dinitrochlorobenzene, 2-nitrochlorobenzene-4-sulfonic acid, or 4-nitrochlorobenzene-2-sulfonic acid.

Substituted toluenes have been carefully nitrated to give nitrophenylmethanes which were then converted into the corresponding benzaldehydes upon treatment with manganese salts in neutral or faintly alkaline solutions (161).

Phenylacetic acids may be oxidized in small yields directly to the corresponding benzaldehydes (148, 159, 266); however, it is better to oxidize them with selenium dioxide in boiling xylene to the phenylglyoxylic acids and then decarboxylate these to the benzaldehydes (30).

E. Oxidation of olefinic side chains

$$C_6H_5CH{=}CHR + \text{oxidant} \rightarrow C_6H_5CHO$$

Olefins, particularly propenylbenzenes, $C_6H_5CH{=}CHCH_3$, are easily converted into benzaldehydes. Chemical methods have been more successful than electrolytic processes. Propenylphenols are the most common type used in this method, since they are conveniently prepared by the allyl rearrangement of the *O*-allyl ethers and subsequent isomerization of the allylphenols to the propenylphenols (3, 286). The most widely employed oxidant and the one which is used for the technical production of substituted *o*- and *p*-hydroxybenzaldehydes is nitrobenzene in dilute alkali (28, 184, 199, 270).

The propenylbenzene, dilute sodium hydroxide solution, and nitrobenzene are mixed, allowed to react for several hours at moderate temperatures with or without pressure, and then the aldehyde is isolated by acidification and steam distillation, or by precipitation.

Several other substances have been used for the oxidation of the propenylbenzenes to aldehydes. Aqueous alkaline peroxides have given only small yields (87). Fair yields may be obtained with anhydrous *tert*-butyl alcohol or *n*-amyl alcohol solutions of hydrogen peroxide, using a catalyst of vanadium pentoxide or chromium trioxide (154). The use of an aqueous alkaline suspension of mercuric oxide in dilute alkali produces a 23 per cent yield of vanillin from eugenol (213). Good yields are attainable by using air with catalysts such as ultraviolet light or terpenes, but the process is slow and has not been developed for general use (46, 80). Sodium dichromate, in the presence of sulfanilic acid, gives 50–70 per cent yields (243). Oxidations with nitric acid, chromyl chloride, or by electrolysis have only resulted in low yields (88, 137, 164, 168, 212, 239).

Ozone, which has been employed by many workers, is not a practical agent for laboratory or technical use, since good yields are often obtained but are not reproducible (270). Hexane or acetic acid solutions of the substituted propenylbenzenes are treated with dilute ozone, whereby an explosive insoluble ozonide is produced, which is then reduced with potassium ferrocyanide or zinc dust in glacial acetic acid (90, 92, 176, 256). By forming an emulsion between the propenylbenzene and aqueous bisulfite tar formation is sometimes minimized and too, the bisulfite addition compound is isolated directly.

Bert has prepared various substituted ω-alkoxypropenylbenzenes in good yields by reacting 1,3-dichloropropene with substituted benzenes in the presence of aluminum chloride and isomerizing the ω-chloroallyl derivatives to cinnamyl ethers by the action of alcoholic potassium hydroxide. He then oxidized these ethers to the substituted benzaldehydes with ozone, a mixture of chromium trioxide plus potassium permanganate, or nitric acid (17).

Stilbenes, cinnamic acids, and other derivatives with olefinic side chains have been oxidized to benzaldehydes in yields which varied and ran as high as 86 per cent when sulfanilic acid was used to remove the aldehyde as it was formed (45, 91, 94, 130, 217, 265).

F. Oxidation of acetophenones

$$C_6H_5COCH_3 + \text{oxidant} \rightarrow C_6H_5COCOOH \rightarrow C_6H_5CHO$$

Acetophenones may be oxidized to phenylglyoxylic acids and these decarboxylated to the corresponding aldehydes by heating in *p*-(*N*,*N*-dimethyl)-

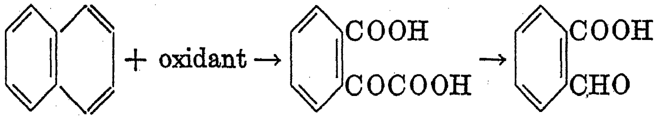
toluidine. This procedure is well illustrated by Mottern's preparation of vanillin from guaiacol (162).

Guaiacol was acetylated, and the ester was subjected to the Fries rearrangement to yield apocynin. This was oxidized with nitrobenzene in dilute alkali to vanilloylformic acid in 85 per cent yield, and the latter was decarboxylated to vanillin (as described above for phenylglyoxylic acids) in 98 per cent yield.

Other authors (244) have not been successful in repeating Mottern's synthesis. Thus, Barch failed to oxidize the apocynin with nitrobenzene to vanilloylformic acid, while Sharygrin and Bogacheva were unable to repeat Mottern's preparation of apocynin or its oxidation to vanilloylformic acid.

A 50 per cent yield of mesitylaldehyde has been obtained from acetylmesitylene by using cold alkaline permanganate (14).

G. Oxidation of naphthalenes



A schematic method for the preparation of substituted *o*-carboxybenzaldehydes is to oxidize a chosen naphthalene derivative to a substituted phthalonic acid and decarboxylate this to the phthalaldehydic acid. The oxidation step is carried out in 40–50 per cent yields, using alkaline permanganate (29, 69, 82, 166, 174), and the resulting phthalonic acids are decarboxylated in 65 per cent yields by boiling in xylene, aqueous bisulfite solutions (33, 174, 279), or as described by Bouveault above (24). Charkravarti and Swaminathan (30) state that derivatives of homophthalic acid are more readily obtained than the required substituted naphthalenes and can be converted into the substituted phthalonic acids with selenium dioxide in xylene in 50–80 per cent yields.

Phthaldehydic acid has also been obtained by the hydrolysis of 2-bromo- or 2-chloro-phthalide, which may be prepared by the halogenation of phthalide (205, 249). The yield of the phthaldehydic acid from the phthalide runs up to 65 per cent.

H. Reduction of acids

$$C_6H_5COOH + \text{reductant} \rightarrow C_6H_5CHO$$

(*1*) *Electrolytic reduction:* The electrolytic reduction of the carboxyl group to the formyl group has been carried out, but the process is not developed to give reproducible results. Mittler (152) reduced benzoic and salicylic acids to the aldehydes in 30–50 per cent yields, using a pool of mercury for a cathode and water–benzene emulsions to dissolve the aldehyde as it is formed. Tesh and Lowy (259) were able to obtain only a 20 per cent yield with this technique. Since the reduction of the aldehyde apparently occurs more easily than the reduction of the acid, some means should always be used to remove the aldehyde as it is produced. To do this Tesh and Lowy tried simultaneous steam distillation, extraction with benzene, or addition of *p*-toluidine, β-naphthylamine, or sodium bisulfite. Using sodium bisulfite in sodium sulfate solutions and a mercury-pool cathode they were able to attain 55 per cent yields of salicylaldehyde from salicylic acid. Cathodes of copper or lead were unsatisfactory, and sodium acetate, sodium chloride, calcium chloride, or potassium sulfate as electrolyte gave poorer results than sodium sulfate. The authors concluded that the process consisted in an electrolytic preparation of sodium amalgam with subsequent chemical reduction. Rutovskii and Korolev (225) repeated Tesh and Lowy's work but obtained only a 34 per cent yield. Kawada and Yosida (116) report an 80 per cent yield of salicylaldehyde from sodium salicylate as a result of a detailed study of electrode material, current density, electrolyte, added reagents, and diaphragm materials. They obtained the best yield when using a mercury-pool cathode, sodium sulfate as the electrolyte, and sodium bisulfite as the agent to remove the aldehyde from solution.

(*2*) *Chemical reduction:* Formic acid, under elevated pressures and temperatures and with the aid of proper catalysts, will reduce carboxylic acids to aldehydes in good yields (44, 225). Davies and Hodgson (44) used titanium dioxide at temperatures of 250–260°C. and obtained yields of aldehydes up to 92 per cent from substituted benzoic acids. If copper or nickel metal is used as the catalyst, further reduction takes place on the side chain or in the ring.

o-Hydroxybenzene- and *o*-hydroxynaphthalene-carboxylic acids are readily reduced to the corresponding aldehydes in 50–60 per cent yields with 2–3 per cent sodium amalgam in the presence of boric acid and a large excess of sodium chloride (280, 281). As in the electrolytic reductions, bisulfite or amine is added to remove the aldehyde as it is formed Reduction will not take place without the addition of boric acid and sodium chloride. Complex salts of boron are thought to be formed between the adjacent hydroxyl and carboxyl groups, since *p*-hydroxybenzoic acids are not reduced in the process (271). Weil, Traun, and Marcel found that polyhydroxybenzoic acids give small yields of aldehydes, if any, while in the case of 5-aminosalicylic acid the amino group is removed from the nucleus and the ring and carboxyl group are reduced (281). Without the addition of an agent to remove the salicylaldehyde as it is formed, salicylic acid gives a 10 per cent yield of *o,o'*-dihydroxystilbene.

Very early Kolbe obtained traces of benzaldehyde from the treatment of benzoic acid with sodium amalgam (121). Baeyer (11) produced small amounts of benzaldehyde by the action of zinc dust on benzoic acid or phthalic acid. Calcium salts of carboxylic acids produce aldehydes when heated with calcium formate, but in the aromatic series only traces of the aldehyde are recovered (138). Thus, vanillin was produced in a 2 per cent yield from vanillic acid (211, 264)

I. Reduction of esters

$$C_6H_5COOR + \text{reductant} \rightarrow C_6H_5CHO$$

The electrolytic and chemical reduction of aromatic esters to aldehydes has shown little success, leading usually to the alcohol. Kharasch, Sternfield, and

Mayo did obtain a 50 per cent yield of benzaldehyde, along with other products, from ethyl benzoate, using sodium dissolved in liquid ammonia (117).

The usual practice is to convert the esters to the acid hydrazides and reduce these as described below.

J. Reduction of acid chlorides

$$C_6H_5COCl + \text{reductant} \rightarrow C_6H_5CHO$$

Baeyer attempted the reduction of acid chlorides to aldehydes with sodium amalgam in acetic acid and isolated only a small amount of the aldehydes, in addition to several other products (12). Since then, others have tried various means of reduction but always with unsatisfactory results (122, 210, 233). Later, Rosenmund succeeded in obtaining high yields, sometimes quantitative, by using hydrogen with a palladium or nickel catalyst (221, 223).

Hydrogen is bubbled through a refluxing solution of the acid chloride and the catalyst until the theoretical amount of hydrogen chloride has been evolved; then the mixture is acidified and the aldehyde isolated by steam distillation (98).

To arrest the reduction at the aldehyde stage, a quinoline-sulfur poison is added. Xylene or tetralin is found to serve best as the solvent (299), while without a solvent the yields are seldom greater than 10 per cent. Hydroxyl groups must be protected; this is usually done by acetylation or carbomethoxylation. Phosphorus- and sulfur-containing side products that are formed in the preparation of the acid chlorides prevent or greatly hinder the reaction, and therefore must be removed (68, 298). Dialdehydes are easily obtainable from diacid chlorides.

Grundman describes a procedure for going from acid chlorides to aldehydes in about 50 per cent yields (84). The acid chlorides are treated with diazomethane

$$C_6H_5COCl + CH_2N_2 \rightarrow C_6H_5COCHN_2 \xrightarrow{CH_3COOH}$$

$$C_6H_5COCH_2OCOCH_3 \rightarrow \underset{\text{(76 per cent yield)}}{C_6H_5CHOHCH_2OH} \xrightarrow{(CH_3COO)_4Pb} \underset{\text{(78 per cent yield)}}{C_6H_5CHO}$$

in a dry solvent, and the products refluxed with acetic acid to split off nitrogen and produce ω-acetoxybenzophenones in 80–90 per cent yields. These are reduced with sodium amalgam or aluminum amalgam in ether or, preferably, with aluminum isopropoxide in isopropyl alcohol, and the substituted β-hydroxy-β-phenylethanols are oxidized with lead tetraacetate to the substituted aldehydes.

Another indirect route of preparing aldehydes from acid chlorides was used by Mauthner, who thereby obtained yields up to 70 per cent (142). He transformed the acid chlorides into the acid cyanides, hydrolyzed the acid cyanides to the phenylglyoxylic acids, and then decarboxylated the latter to the aldehydes by heating them in aniline.

K. Reduction of nitriles

$$C_6H_5CN + HCl \rightarrow C_6H_5CCl{=}NH\cdot HCl + \text{reductant} \rightarrow C_6H_5CHO$$

Stephen worked out a method for preparing aldehydes from nitriles in yields that are usually greater than 90 per cent (258, 289). Hydrogen chloride is bubbled through a dry ether solution of the nitrile and anhydrous stannous chloride during a period of several hours. The resulting imidochloride may be isolated, or reduced directly to the aldehyde. If necessary, chloroform may be added to the ether to aid solution of the nitrile.

The reaction is general except for steric hindrance due to groups ortho to the nitrile group. Thus, α-naphthonitrile, *o*-tolunitrile, and *o*-nitrobenzonitrile give negligible yields of the aldehydes (258, 288, 289). Nitro groups do not interfere with the success of the reaction; however, they may be reduced if sufficient stannous chloride is used.

Another method for reducing benzonitriles to the corresponding aldehydes is that of Henle, which gives yields up to 95 per cent (95). It consists in converting the nitriles into the imido-esters, reducing the latter with sodium amalgam in the presence of phenylhydrazine, and hydrolyzing the resulting phenylhydrazones to liberate the aldehydes. Semicarbazide or substituted hydrazines may be used in place of phenylhydrazine, but aniline gives rise to many side products; without the addition of the amines the yields are very low. Other reducing agents, such as zinc dust, tin, stannous chloride, or magnesium, are unsatisfactory.

L. Reduction of amides and imidochlorides

$$\begin{tikzcd} C_6H_5CONR_2 + \text{reductant} \arrow[dr] & \\ & C_6H_5CHO \\ C_6H_5CCl{=}NH\cdot HCl + \text{reductant} \arrow[ur] & \end{tikzcd}$$

The direct reduction of amides to aldehydes has always given poor results (85, 107, 290), the best yield being only 20–30 per cent (150). An alternative scheme for changing amide groups into formyl groups is through the reduction of the imidochloride which is prepared from the amide by treatment with phosphorus pentachloride or thionyl chloride. Staudinger (257) used magnesium in ethyl acetate to reduce the imidochloride and obtained 40–50 per cent yields. Later, Sonn and Muller reported the method of bubbling hydrogen chloride through an ether solution of the imidochloride and anhydrous stannous chloride to reduce the imidochloride and then hydrolyzing the product to isolate the aldehyde (255). Over-all yields from the amides averaged 50–60 per cent. Hydroxyl groups are protected by carbomethoxylation. As in Stephen's reaction above, groups ortho to the imidochloride group hinder the reaction. Anhydrous chromous chloride may be substituted for the stannous chloride, but it is more difficult to prepare and, unless it is dry, hydrolysis of the imidochloride takes place faster than its reduction (26).

M. Reduction of hydrazides

$$C_6H_5COOR \rightarrow C_6H_5CONHNH_2 \rightarrow C_6H_5CONHNHSO_2C_6H_5 \rightarrow C_6H_5CHO$$

As stated above, esters are not reduced to aldehydes directly but may be converted into their hydrazides, which may then be reduced to the aldehydes.

McFadyen and Stevens (147) worked out a general procedure, giving 50–65 per cent yields, and the method is found to apply to most substituted benzhydrazides (93, 165, 167, 269).

The ester is converted into the hydrazide, which is then treated with benzenesulfonyl or *p*-toluenesulfonyl chloride, and the product is decomposed by heating with sodium carbonate in ethylene glycol at 160°C. to produce the aldehyde.

According to Kalb and Gross (112), acid hydrazides react in three ways:

$$(1) \quad RCONHNH_2 + O_2 \rightarrow RCOOH + H_2O + N_2$$

$$(2) \quad 2RCONHNH_2 + O_2 \rightarrow RCONHNHCOR + H_2O + N_2$$

$$(3) \quad 2RCONHNH_2 + \tfrac{1}{2}O_2 \rightarrow RCH{=}NNHCOR \rightarrow 2RCHO + H_2O + N_2$$

As indicated in equations 2 and 3, Curtius and Struve (41) oxidized benzhydrazide with iodine or mercuric oxide to produce dibenzoylhydrazine and benzalbenzoylhydrazine, and Darapsky (42) used sodium hypochlorite to obtain a low yield of benzaldehyde. Kalb and Gross tried several oxidants and found that, by treating various substituted benzhydrazides with potassium ferricyanide in excess ammonium hydroxide, the corresponding aldehydes are produced in yields up to 65 per cent.

N. Aldehydes from Grignard reagents

At least ten types of compounds which react with aromatic Grignard reagents to produce aldehydes are recorded in the literature. These include hydrogen cyanide, alkyl orthoformates, formate esters, formic acid, copper formate, disubstituted formamides, methyl isocyanide, ethoxymethyleneaniline, carbon disulfide, and chloral.

Wuyts developed the method of using carbon disulfide. The Grignard reagents are treated with carbon disulfide to produce the dithio acids, these are digested with a pyridine solution of semicarbazide hydrochloride, and the resulting semicarbazone is hydrolyzed to liberate the aldehyde (294, 295, 296).

$$C_6H_5MgX + CS_2 \rightarrow C_6H_5CSSH \rightarrow C_6H_5CH{=}NNHCONH_2 \rightarrow C_6H_5CHO$$

When phenylhydrazine or hydroxylamine was tried in place of the semicarbazide, several side reactions were found to occur. Wuyts's only mention of the yield from the aromatic halide was for *p*-dibromobenzene, in which case the yield was 46 per cent. The yields based on the amount of aldehyde produced from the dithio acid average about 80 per cent.

Savariau reacted chloral with Grignard reagents to produce phenyltrichloromethylcarbinols, hydrolyzed these with aqueous alkali to liberate mandelic acids, and decarboxylated the latter by heating them with dilute potassium carbonate, producing the substituted benzaldehydes (232).

Sachs and Loewy (229) treated Grignard reagents with methyl isocyanide to produce aldehydes; later, Gilman and Hechert found that of the alkyl isocyanides, only methyl isocyanide will serve for the reaction (81). Hinkel and Dunn prepared benzaldehyde by addition of phenylmagnesium bromide to an ether solution of bimolecular hydrogen cyanide and subsequent hydrolysis (100). Methyl and amyl formates (15), formic acid (103, 297), and copper formate (103) have been used to convert Grignard reagents into aldehydes in yields of 30 per cent or less.

Bouveault introduced the use of aryl- or alkyl-disubstituted formamides to convert Grignard reagents to aldehydes in yields varying between 30 and 70 per cent (24, 25).

$$C_6H_5MgX + HCONRR' \rightarrow C_6H_5CH(OMgX)NRR' \rightarrow C_6H_5CHO$$

It is found that, depending upon the substituted amide, the reaction follows one of two different courses (105, 144, 145, 272):

$$(1) \qquad HCONR_2 + C_6H_5MgX \rightarrow C_6H_5CHO + R_2NH$$
$$(2) \qquad HCONR_2 + 2C_6H_5MgX \rightarrow (C_6H_5)_2CHNR_2$$

Bodroux (21), Tschitschibabin and Gattermann (267), and Maffezzoli (76) independently observed that ethyl orthoformate reacts with Grignard reagents to produce aldehydes and secondary alcohols.

$$C_6H_5MgX + HC(OC_2H_5)_3 \rightarrow C_6H_5CH(OC_2H_5)_2 \rightarrow C_6H_5CHO$$

If excess ethyl orthoformate is used, the product consists mainly of the aldehyde. The pure acetal may be recovered by fractionation or hydrolyzed directly to the aldehyde. Methyl orthoformate has also been used to convert Grignard reagents to the corresponding aldehyde in 55–70 per cent yields (228). Yields reported by different authors varied so widely that Smith and Bayliss experimented to determine the conditions for obtaining the maximum yield, which averaged about 60–70 per cent (251).

Monier and Williams obtained aldehydes from Grignard reagents in yields of 30–60 per cent by using ethoxymethyleneaniline (160).

$$C_6H_5MgX + C_6H_5N{=}CHOC_2H_5 \rightarrow C_6H_5CH{=}NC_6H_5 \rightarrow C_6H_5CHO$$

Of the above methods, Smith and Nichols assumed that those which use ethyl orthoformate, ethoxymethyleneaniline, and carbon disulfide give the most reproducible results and proceeded to compare these three (252). Their results indicated that carbon disulfide, ethyl orthoformate, and ethoxymethyleneaniline give increasing yields, respectively; owing to the unavailability of the last substance, they concluded that ethyl orthoformate is the best reagent to use with Grignard reagents for the preparation of aldehydes.

O. *Aldehydes from benzophenone oxime*

$$(C_6H_5)_2C{=}NOH + P_2S_5 \rightarrow C_6H_6CSNHC_6H_5 \rightarrow C_6H_5CONHC_6H_5 \rightarrow C_6H_5CHO$$

Cuisa (39) used a unique procedure of obtaining benzaldehyde from benzophenone oxime by refluxing it with phosphorus pentasulfide in carbon disulfide, boiling the resulting thiobenzanilide in aqueous alkaline silver nitrate to produce benzanilide, and treating the latter with zinc dust in 20 per cent potassium hydroxide solution. He isolated benzaldehyde in a 42 per cent over-all yield.

TABLE 2

Yields in the preparation of aldehydes

METHOD	HALOGEN	NITRO	SULFO	CYANO	HYDROXYL	ALKOXYL	ALKYL OR HYDROGEN	POLYCYCLIC	ACYL	AMINO
Gattermann and Koch reaction							60–80			
Gattermann reaction					50–100	70–100	75–85	60–90		
Reimer–Tiemann reaction					30–50			50–80		
Reactions with hexamethylenetetramine					15–20			20–25		35–45
Reactions with *N*-methylformanilide						70–85		60–90		50–80
Reactions with formaldehyde					40–50			40–60		40–50
Reactions with chloral					40–60					
Reactions with alloxan	Yields were not located									
Reactions with mesoxalates					50–80					50–80
Reactions with oxalyl ester acid chlorides					40–60	40–60	40–60			
Reactions with sodium glyoxylate					50–70					
Reactions with mercury fulminate					60–80		50–60			
Reactions with chloromethylene dibenzoate						60–80	30–50			
Reactions with isatin chloride					30			30		
Reactions with organolithium compounds						50–60	50–60			
Naphthaldehydes from benzofurans and acrolein								50		
Oxidation of saturated side chains	50									80
Oxidation of benzyl alcohols	60									90
Oxidation of benzyl halides	50									80
Oxidation of benzylanilines, benzylamines, *N*-alkylanilines, phenylnitromethanes, and phenylacetic acids	30									50
Oxidation of olefinic side chains	50									70
Oxidation of acetophenones	Method not standardized									
Oxidation of naphthalenes	25									35
Reduction of acids					50–60		50–90	50–90		
Reduction of esters	Method not standardized									
Reduction of acid chlorides	60–90	40–50				60–90	60–90	60–90	60–90	
Reduction of nitriles	60									90
Reduction of amides and imidochlorides	50									60
Reduction of hydrazides	50									65
Aldehydes from Grignard reagents	30									50

IV. NON-PREPARATIVE REACTIONS

There are a number of reactions by which small amounts of aldehydes are produced, but they should not be considered as preparative methods. Among these are the following:

Benzil, when reacted with alcoholic potassium cyanide, produces the ester of benzoic acid and benzaldehyde (111).

Benzyl sulfoxide, when treated with a chloroform solution of hydrogen chloride, produces traces of benzaldehyde (97).

Aldehydes have been isolated by refluxing phenols with ethyl orthoformate and a non-aqueous base and hydrolyzing the acetal produced with mineral acid. Upon refluxing *o*-hydroxyphenylmercuric chloride with ethyl orthoformate and then hydrolyzing the product in dilute acid, a small amount of salicylaldehyde is obtained (271).

Aluminum chloride-catalyzed condensations of substituted benzenes with alkyl and aryl carboxylic acids produce derivatives of acetophenone and benzophenone, respectively, in good yields. In accordance with this, formic acid should lead to the aldehyde but only a 1–2 per cent yield has been attained (83). Probably the aldehyde reacts further as soon as it is formed.

Aldehydes are among the pyrolysis products of several compounds (19, 66, 67, 119, 126, 135).

Electrolytic processes are known to yield traces of aldehydes (13, 27, 155, 277).

V. YIELDS IN THE SYNTHESIS OF ALDEHYDES

In table 2 are listed the average per cent yields that may be expected from the different methods when the various substituents listed at the top are attached to the benzene ring. Under the heading "polycyclic" are listed the yields from any of the methods when applied to polycyclic compounds in general, without regard to substituents. Methods which can be employed only for phenol and its substitution products are listed under the column headed "hydroxyl." With this exception, blank spaces indicate that the method is not recommended in the case of such substituents. Extended yields imply that the method is general and inapplicable only where obvious chemical reactions will occur between the reactants and the substituents.

The author wishes to acknowledge his indebtedness to Professor Melvin Calvin, of the University of California, whose numerous suggestions were followed in carrying out investigations of several of the methods described in this paper, and also to Professor R. Percy Barnes, of Howard University, for his kindness in reviewing the manuscript.

VI. REFERENCES

(1) Adams, R.: J. Am. Chem. Soc. **45**, 2373 (1923).

(2) Adams, R.: J. Am. Chem. Soc. **46**, 1518 (1924).

(3) Adams, R.: *Organic Reactions*, Vol. II. John Wiley and Sons, Inc., New York (1944).

(4) Aleksandrova, Z. P.: J. Applied Chem. (U. S. S. R.) **10**, 105 (1937).
(5) Angeli, A., and Angelico, F.: Chem. Zentr. **1899**, ii, 371.
(6) Appelbaum, A.: U. S. patent 1,302,273 (1919).
(7) Armstrong, D. E., and Richardson, D. H.: J. Chem Soc. **1933**, 496.
(8) Arnold, R., and Sprung, J.: J. Am. Chem. Soc. **60**, 1699 (1938).
(9) Aston, G., Newman, C., and Riley, H.: J. Chem. Soc. **1933**, 391.
(10) Auwers, K., and Keil, G.: Ber. **35**, 4207 (1902); **36**, 1861 (1903).
(11) Baeyer, A.: Ann. **140**, 295 (1866).
(12) Baeyer, A.: Ber. **2**, 98 (1869).
(13) Baillie, T., and Tafel, J.: Ber. **32**, 68 (1899).
(14) Barnes, R. P., Pierce, Charles I., and Cochrane, Chappelle C.: J. Am. Chem. Soc. **62**, 1084 (1940).
(15) Bayer and Company: J. Chem. Soc. **88**, 355 (1905).
(16) Beard, H., and Hodgson, H.: J. Chem. Soc. **1944**, 4.
(17) Bert, L.: Compt. rend. **215**, 187 (1942).
(18) Bezdzik, A., and Friedlaender, P.: Monatsh. **30**, 271 (1909).
(19) Bischoff, C. A., and Walden, P.: Ann. **279**, 118 (1894).
(20) Blanc, G.: British patent 115,244 (1918).
(21) Bodroux, F.: Compt. rend. **138**, 92, 700 (1904).
(22) Bohringer, C. F., und Sohne: German patents 107,720 and 115,817 (1901).
(23) Bornemann, E.: Ber. **17**, 1462 (1884).
(24) Bouveault, L.: Bull. soc. chim. [3] **15**, 1014 (1896); **17**, 363, 940, 943 (1897).
(25) Bouveault, L.: Compt. rend. **137**, 987 (1903).
(26) Braun, J. von, and Rudolph, W.: Ber. **67**, 269 (1934).
(27) Brockman, C. J.: *Electro-Organic Chemistry*. John Wiley and Sons, Inc., New York (1926).
(28) Carroll, M., and Boake, A.: British patent 417,072 (1934).
(29) Chakravarti, S. N.: J. Indian Chem. Soc. **10**, 693 (1933).
(30) Chakravarti, S. N., and Swaminathan, M.: J. Indian Chem. Soc. **11**, 715, 813 (1934); **17**, 264 (1940); **20**, 382 (1943).
(31) Chaudhuri, T. C.: J. Am. Chem. Soc. **64**, 315 (1942).
(32) Chelintzev, G., and Rodnevich, B.: J. Applied Chem. (U. S. S. R.) **8**, 909 (1935).
(33) Claisen, L.: Ber. **10**, 429, 1663 (1877).
(34) Cohen, J., and Calvert, H.: J. Chem. Soc. **71**, 1051 (1897).
(35) Cohn, P., and Friedlander, P.: Ber. **35**, 1265 (1902).
(36) Coles, H. W., and Dodds, M. L.: J. Am. Chem. Soc. **60**, 853 (1938).
(37) Coninck, O., and Combe, A.: Compt. rend. **127**, 1221 (1898).
(38) Copisarow, M.: J. Chem. Soc. **1929**, 588.
(39) Cuisa, R.: Chem. Zentr. **1907**, i, 28.
(40) Curme, H. R.: Canadian patent 238,730 (1924).
(41) Curtius, Th., and Struve, G.: J. prakt. Chem. [2] **50**, 300 (1894); **81**, 501 (1910); Ber. **33**, 2559 (1900).
(42) Darapsky, A.: Ber. **40**, 3033 (1907).
(43) Davies, R. R., and Hodgson, H. H.: J. Soc. Chem. Ind. **62**, 109 (1943).
(44) Davies, R. R., and Hodgson, H. H.: J. Chem. Soc. **1943**, 84, 281.
(44a) Davies, R. R., and Hodgson, H. H.: J. Chem. Soc. **1943**, 282.
(45) Davies, R. R., and Hodgson, H. H.: J. Soc. Chem. Ind. **62**, 90 (1943).
(46) Delapierre, F.: German patent 150,981 (1904).
(47) Dippy, J. F., *et al.*: J. Soc. Chem. Ind. **56**, 346 (1937).
(48) Duff, J.: J. Chem. Soc. **1941**, 547.
(49) Duff, J.: J. Chem. Soc. **1945**, 276.
(50) Duff, J., and Bills, E.: J. Chem. Soc. **1932**, 1987; **1934**, 1305.
(51) Endraszka, J.: Kron. Farm. **38**, 101 (1939).
(52) Étard, A.: Ann. chim. phys. [5] **22**, 223 (1881).

(53) Étard, A.: Compt. rend. **90,** 534 (1880).
(54) Fichter, F., and Basil: Z. Elektrochem. **19,** 781 (1913).
(55) Fichter, F., and Stocker, R.: Ber. **47,** 2003 (1914).
(56) Fieser, L. F., and Hartwell, J.: J. Am. Chem. Soc. **60,** 2555 (1938).
(57) Fieser, L. F., Hartwell, J. L., and Jones, J. E.: Org. Syntheses **20,** 11 (1940).
(58) Fieser, L. F., and Hershberg, E.: J. Am. Chem. Soc. **60,** 2542 (1938).
(59) Fieser, L. F., and Jones, J. E.: J. Am. Chem. Soc. **64,** 1666 (1942).
(60) Fischer, O., and Greiff, P.: Ber. **13,** 669 (1880).
(61) Fisher, C.: J. Am. Chem. Soc. **56,** 2056 (1934).
(62) Fisher, C., and Eisner, A.: J. Org. Chem. **6,** 169 (1940).
(63) Francesconi, L., and Mundici, C.: J. Chem. Soc. **84,** 426 (1903); **86,** 897 (1904).
(64) Freund, R.: Chem.-Ztg. **51,** 803 (1927).
(65) Friedlaender, P.: Ber. **41,** 1035 (1908).
(66) Fromm, E., and Achert, O.: Ber. **36,** 534 (1903).
(67) Fromm, E., and Palma, J.: Ber. **39,** 3308 (1906).
(68) Fröschl, N., and Danoff, C.: J. prakt. Chem. **144,** 217 (1936).
(69) Gardner, J.: J. Indian Chem. Soc. **11,** 401 (1934).
(70) Gattermann, L.: Ann. **347,** 347 (1906); **357,** 313 (1907).
(71) Gattermann, L.: Ber. **31,** 1149 (1898).
(72) Gattermann, L., and Berchelmann, W.: Ber. **31,** 1765 (1898).
(73) Gattermann, L., and Horlacher, Th. von: Ber. **32,** 284 (1899).
(74) Gattermann, L., and Köbner, M.: Ber. **32,** 278 (1899).
(75) Gattermann, L., and Koch, J.: Ber. **30,** 1622 (1897).
(76) Gattermann, L., and Maffezzoli, F.: Ber. **36,** 4152 (1903); Ann. **393,** 215 (1912).
(77) Geigy, J.: German patent 103,578 (1899).
(78) Geigy, J.: German patents 105,103 (1900); 105,798 (1900).
(79) Geneff, C.: U. S. patent 2,154,979 (1939).
(80) Genthe and Co.: German patents 225,265 (1910) and 224,071 (1910).
(81) Gilman, H., and Heckert, L.: Bull. soc. chim. [4] **43,** 224 (1928).
(82) Graebe, C., and Trumpy, F.: Ber. **31,** 369 (1898).
(83) Groggins, P. H., Nagel, R. H., and Stirton, A. J.: Ind. Eng. Chem. **26,** 1317 (1934).
(84) Grundmann, C.: Ann. **524,** 31 (1936).
(85) Guareschi, J.: Ber. **7,** 1462 (1874).
(86) Gugyot, A., and Gry, A.: Bull. soc. chim. [4] **7,** 902 (1910).
(87) Haarmann and Reimer: German patent 93,938 (1897).
(88) Haarmann and Reimer: J. Soc. Chem. Ind. **14,** 506 (1895).
(89) Hanus, F.: J. prakt. Chem. **158,** 254 (1941).
(90) Harries, C.: German patent 321,567.
(91) Harries, C.: Ber. **36,** 1933 (1903).
(92) Harries, C., and Haarmann, R.: Ber. **48,** 32 (1915).
(93) Harington, C., and Rivers, R.: J. Chem. Soc. **1940,** 1101.
(94) Henderson, G., and Gray, T.: J. Chem. Soc. **85** 1041 (1904).
(95) Henle, F.: Ber. **35,** 3039 (1902); **38,** 1362 (1905).
(96) Henry T., and Sharp, T.: J. Chem. Soc. **1926,** 2432.
(97) Herrmann, F.: Ber. **39,** 3812 (1906).
(98) Hershburg, E. B., and Cason, J.: Org. Syntheses **21,** 84 (1941).
(99) Hey, D.: J. Chem. Soc. **1935,** 72.
(100) Hinkel, L., and Dunn, R.: J. Chem. Soc. **1930,** 1834.
(101) Hodgson, H., and Jenkinson, T.: J. Chem. Soc. **1927,** 1740; **1929,** 469.
(102) Holloway, J., and Krase, N.: Ind. Eng. Chem. **25,** 497 (1933).
(103) Houben, J.: Chem.-Ztg. **29,** 667 (1905).
(104) Houben, J.: Ber. **59,** 2878 (1926).
(105) Houben, J., and Doescher, H.: Ber. **40,** 4576 (1907).

(106) Hurd, C. D.: *The Pyrolysis of Carbon Compounds*, A.C.S. Monograph No. 50. Reinhold Publishing Corporation, New York (1929).
(107) Hutchinson, A.: Ber. **24**, 173 (1891).
(108) Jaeger, A.: U. S. patent 1,909,354 (1933).
(109) James, J.: J. Am. Chem. Soc. **21**, 889 (1899).
(110) Jones, J. E.: Am. Perfumer **46**, No. 3, 37 (1944).
(111) Jourdan, F.: Ber. **16**, 658 (1883).
(112) Kalb, L., and Gross, O.: Ber. **59**, 727 (1926).
(113) Kalischer, G., and Keller, K.: German patent 519,806 (1928).
(114) Kalischer, G., Scheyer, H., and Keller, K.: German patent 514,415 (1927).
(115) Kawada, G.: J. Pharm. Soc. Japan, No. **521**, 628 (1925).
(116) Kawada, G., and Yosida, J.: Bull. Hyg. Research Inst. (Japan) **35**, 261 (1929).
(117) Kharasch, M., Sternfield, E., and Mayo, F.: J. Org. Chem. **5**, 363 (1940).
(118) Kirkhyof, G., Rosina, S., and Butyugin, S.: Russian patent 37,704.
(119) Knoevenagel, E., and Tomasczewski, A.: Ber. **36**, 2829 (1903).
(120) Koebner, A., and Robinson, R.: J. Chem. Soc. **1938**, 1994.
(121) Kolbe, H.: Ann. **118**, 122 (1861).
(122) Kolbe, H.: J. prakt. Chem. [2] **4**, 418 (1871).
(123) Korczynski, A., and Mrozinski, W.: Bull. soc. chim. [4] **29**, 459 (1921).
(124) Krishkov, A.: Org. Chem. Ind. (U. S. S. R.) **5**, 551 (1938).
(125) Kuznetzov, M., and Stepanenko, M.: Ukraïn. Khem. Zhur. **4**, Tech. Pat. 153 (1929).
(126) Lachman, A.: J. Am. Chem. Soc. **45**, 2356 (1923).
(127) Larson, A.: U. S. patent 1,989,700 (1935).
(128) Law, H., and Perkin, F.: Trans. Faraday Soc. **1**, 31, 251 (1905); Chem. News **92**, 68 (1905).
(129) Law, H., and Perkin, F.: J. Chem. Soc. **91**, 258 (1907).
(130) Levinstein: German patents 119,163 and 115,410 (1901).
(131) Liggett, L. M.: Iowa State Coll. J. Sci. **19**, 44 (1944).
(132) Limpricht, H.: Ann. **144**, 304 (1867).
(133) Lindemann, H.: Ann. **431**, 270 (1923).
(134) Lorges, B.: Rev. chim. ind. **34**, 10, 50 (1925).
(135) Lowe, C.: Ann. **241**, 374 (1887).
(136) Lowy, A., and Moore, C.: Trans. Am. Electrochem. Soc. **42**, 273 (1922).
(137) Lucius and Bruning: German patent 107,095 (1900).
(138) Ludewig: Ann. **379**, 352 (1911).
(139) Makarov-Zemlyanskiĭ, Ya, *et al.*: J. prakt. Chem. **147**, 317 (1937).
(140) Mann, C., and Paulson, P.: Trans. Am. Electrochem. Soc. **47**, 101 (1925).
(141) Marek, L. F., and Hahn, D. A.: *Catalytic Oxidation of Organic Compounds in Vapor Phase*, A.C.S. Monograph No. 61. The Chemical Catalog Company, Inc., New York (1932).
(142) Mauthner, F.: Ber. **41**, 920 (1908); **42**, 188 (1909).
(143) Margolis, L.: J. Applied Chem. (U. S. S. R.) **14**, 827 (1941).
(144) Maxim, N.: Bull. soc. chim. [4] **41**, 809 (1927).
(145) Maxim, N., and Mavrodineanu, R.: Bull. soc. chim. [5] **2**, 591 (1935); **3**, 1084 (1936).
(146) Maxted, E.: J. Soc. Chem. Ind. **47**, 101 (1928).
(147) McFadyen, J., and Stevens, T.: J. Chem. Soc. **1936**, 584.
(148) Meissner, C.: German patent 17,107.
(149) Meister, Lucius, and Bruning: British patents 10,689, 30,118; German patent 110,173; J. Soc. Chem. Ind. **16**, 558 (1897); **18**, 36 (1899).
(150) Merling, G.: Ber. **41**, 2064 (1908).
(151) Merzbacher, A., and Smith, E.: J. Am. Chem. Soc. **22**, 723 (1900).
(152) Mettler, C.: Ber. **41**, 4148 (1908).
(153) Michaelis, A., and Storbech, O.: Ann. **274**, 197 (1893).

(154) Milas, N.: J. Am. Chem. Soc. **59**, 2342 (1937).
(155) Miller, W., and Hofer, J.: Ber. **27**, 461 (1894).
(156) Miller, W., and Rohde, G.: Ber. **23**, 1070 (1890).
(157) Mitchell, R.: Trans. Am. Electrochem. Soc. **56**, 495 (1929).
(158) Mittal, D. S.: J. Indian Chem. Soc. **19**, 408 (1942).
(159) Möller, F., and Strecker, A.: Ann. **113**, 56 (1860).
(160) Monier-Williams, G. W.: J. Chem. Soc. **89**, 273 (1906).
(161) Monnet, P., and Cartier: German patents 239,953 and 237,358 (1911).
(162) Mottern, H.: J. Am. Chem. Soc. **56**, 2107 (1934).
(163) Moureu, C., and Mignonac, G.: Compt. rend. **171**, 652 (1920).
(164) Nachfolger, F.: German patent 92,007 (1897).
(165) Natelson, S., and Gottfried, S.: J. Am. Chem. Soc. **63**, 487 (1941).
(166) Naylor, C., and Gardner, J.: J. Am. Chem. Soc. **53**, 4109 (1931).
(167) Niemann, C., and Hays, J. T.: J. Am. Chem. Soc. **65**, 482 (1943).
(168) Novarine, J.: U. S. patents 365,918 and 365,919; Ber. **29**, Ref. 812 (1896).
(169) Oakwood, T. S., Wagner, R. B., Bassler, G. C., Krimmel, J. A., and Ashcraft, J. O.: Paper presented before the Division of Organic Chemistry at the 108th Meeting of the American Chemical Society, New York, September, 1944.
(170) *Organic Syntheses*, Collective Vol. II, p. 441. John Wiley and Sons, Inc., New York (1943).
(171) Reference 170, p. 583.
(172) Reference 170, pp. 89, 133.
(173) Reference 170, p. 223.
(174) Reference 170, p. 523.
(175) *Organic Syntheses*, Collective Vol. I, p. 214. John Wiley and Sons, Inc., New York (1941).
(176) Otto, M., and Verley, A.: German patent 97,620 (1898).
(177) Patents: British 311,208 (1928).
(178) Patents: British 161,679 (1921).
(179) Patents: British 401,562 (1933).
(180) Patents: British 189,091 (1923); 189,107 (1923).
(181) Patents: British 399,723 (1933).
(182) Patents: British 294,889 (1927).
(183) Patents: British 250,955 (1925).
(184) Patents: British 392,399; 285,451 (1928).
(185) Patents: French 791,818 (1935).
(186) Patents: French 751,687 (1933).
(187) Patents: French 653,975 (1928).
(188) Patents: French 648,069 (1928).
(189) Patents: German 403,489 (1924).
(190) Patents: German 598,652 (1934); 475,918 (1923).
(191) Patents: German 162,322 (1905).
(192) Patents: German 127,388; 158,609 (1901).
(193) Patents: German 121,785 (1901).
(194) Patents: German 86,874; 87,255 (1896).
(195) Patents: German 121,745 (1901).
(196) Patents: German 268,786 (1914).
(197) Patents: German 91,503; 92,084 (1897).
(198) Patents: German 157,573 (1905).
(199) Patents: Japanese 109,457 (1935).
(200) Patents: Swiss 181,807 (1936).
(201) Patents: U. S. 1,939,005 (1934).
(202) Patents: U. S. 1,636,854 (1927); 1,321,959 (1919); 1,937,962 (1934).
(203) Patents: U. S. 1,965,458 (1935).

(204) Patents: U. S. 1,405,261 (1922).
(205) Patents: U. S. 2,047,946 (1936).
(206) Patents: U. S. 1,487,020 (1924).
(207) Patents: U. S. 1,807,693 (1933).
(208) Paterno, E., and Scichilone, S.: Ber. **14**, 525 (1881).
(209) Pauly, H., and Schanz, H.: Ber. **56**, 979 (1923).
(210) Perkin, W. H. and Sudborough, J. J.: Proc. Chem. Soc. **1894**, p. 216.
(211) Piria, R.: Ann. **100**, 105 (1856); Ann. chim. phys. [3] **48**, 113 (1856).
(212) Preu, O.: Ber. **29**, Ref. 813 (1896).
(213) Pum, G.: German patent 92,466 (1897).
(214) Putnam, M.: U. S. patent 1,272,522 (1918).
(215) Rapson, W. S., Saunder, D. H., and Stewart, E. T.: J. Chem. Soc. **1944**, 74.
(216) Reimer, K., and Tiemann, F.: Ber. **9**, 824 (1876).
(216a) Reissert, A.: Ber. **40**, 4209 (1907).
(217) Reissert, A., and Scherk, J.: Ber. **31**, 387 (1898).
(218) Renard, A.: Compt. rend. **91**, 175 (1880); **92**, 965 (1881).
(219) Richter, V.: Ber. **19**, 1060 (1886).
(220) Richter, V., and Schüchner, G.: Ber. **17**, 1931 (1884).
(221) Riedel, J., and Haen, E.: German patents 617,763 and 621,567 (1935); British patent 401,562 (1933).
(222) Rosenmund, K.: Ber. **51**, 585 (1918).
(223) Rosenmund, K., and Zetzsche, F.: Ber. **51**, 594 (1918); Ber. **54**, 425, 2885 (1921); **55**, 609 (1922).
(224) Russell, A., and Lockhart, L. B.: Org. Syntheses **22**, 63 (1942).
(225) Rutovskii, B., and Korolev, A.: Trans. Sci. Chem. Pharm. Inst. (U. S. S. R.) **19**, 177 (1928).
(226) Sabatier, P., and Mailhe, A.: Compt. rend. **154**, 561 (1912); **158**, 985, 830 (1914).
(227) Sachs, A.: Perfumers' J. **7**, No. 3, 11, 32 (1926).
(228) Sachs, F., and Kempf, R.: Ber. **35**, 1224 (1902).
(229) Sachs, F., and Loevy, H.: Ber. **37**, 874 (1904).
(230) Sah, P. P. T.: Rec. trav. chim. **59**, 461 (1940).
(231) Sah, P. P. T.: Rec. trav. chim. **60**, 373 (1941).
(232) Savariau: Compt. rend. **146**, 297 (1908).
(233) Saytzeff, A.: Ann. **171**, 258 (1874).
(234) Schaack, R.: U. S. patent 2,261,193 (1942).
(235) Scholl, R.: Ber. **32**, 3492 (1899).
(236) Scholl, R., and Kacer, F.: Ber. **36**, 322 (1903).
(237) Schulze, K.: Ber. **17**, 1530 (1884).
(238) Schwyzer, J.: Chem.-Ztg. **54**, 839 (1930).
(239) Schwyzer, J.: Chem.-Ztg. **54**, 817, 839 (1930).
(240) Segesser, J.: and Calvin, M.: J. Am. Chem. Soc. **64**, 825 (1942).
(241) Sen, R., and Ray, S.: J. Indian Chem. Soc. **9**, 173 (1932).
(242) Shah, R., Shah, H., and Laiwalla, M.: J. Chem. Soc. **1938**, 1828; **1939**, 132, 300.
(243) Shamshurin, A. A.: J. Gen. Chem. (U. S. S. R.) **14**, 211 (1944).
(244) Shorygrin, P., and Bogacheva, K.: J. Gen. Chem. (U. S. S. R.) **6**, 1567 (1926). Barch, W.: J. Am. Chem. Soc. **57**, 2330 (1935).
(245) Shoruigin, P. P., *et al.*: J. Chem. Ind. (U. S. S. R.) **6**, 258 (1929).
(246) Shoruigin, P. P., Kizber, I., and Smol. Yaninova, E.: Zhur. Priklad. Khim. **3**, 721 (1930).
(247) Shoruigin, P. P., and Losev, I.: J. Gen. Chem. (U. S. S. R.) **3**, 821 (1933).
(248) Shoruigin, P. P., and Sokolova, A. M.: J. Russ. Phys. Chem. Soc. **62**, 673 (1930).
(249) Shriner, R. L., and Wolf, F. J.: Org. Syntheses **23**, 74 (1943).
(250) Smith, L. I., *et al.*: J. Org. Chem. **4**, 323 (1939).
(251) Smith, L. I., and Bayliss, M.: J. Org. Chem. **6**, 437 (1941).

(252) Smith, L. I., and Nichols, J.: J. Org. Chem. **6**, 489 (1941).
(253) Snell, J. M., and Weissberger, A.: Org. Syntheses **20**, 92 (1940).
(254) Sommelet, M.: Compt. rend. **157**, 852 (1913).
(255) Sonn, A., and Müller, E.: Ber. **52**, 1927 (1919).
(256) Spurge, E.: German patent 192,565 (1908).
(257) Staudinger, H.: Ber. **41**, 2217 (1908).
(258) Stephen, H.: J. Chem. Soc. **127**, 1874 (1925).
(259) Tesh, K., and Lowy, A.: Trans. Am. Electrochem. Soc. **45**, 37 (1924).
(260) Thiele, J., and Winter, E.: Ann. **311**, 353 (1900).
(261) Thiele, J., and Eichwede, H.: Ann. **311**, 363 (1900).
(262) Thomas, C. A.: *Anhydrous Aluminum Chloride in Organic Chemistry*, A.C.S. Monograph No. 87. Reinhold Publishing Corporation, New York (1941).
(263) Thompson, H. W.: J. Chem. Soc. **1932**, 2310.
(264) Tiemann, F.: Ber. **8**, 1129 (1875).
(265) Tiemann, F., *et al.*: Ber. **9**, 409 (1876); **11**, 646 (1878); **7**, 608 (1874); **18**, 3481 (1885).
(266) Tiemann, F., and Nagai, N.: Ber. **10**, 201 (1877).
(267) Tschitschibabin, A.: J. Russ. Phys. Chem. Soc. **35**, 1284 (1903); Ber. **37**, 186, 850 (1904); Ber. **44**, 447 (1911).
(268) Ullmann, F., and Frey, B.: Ber. **37**, 855 (1904).
(269) Ungnade, H.: J. Am. Chem. Soc. **63**, 2091 (1941).
(270) Unpublished correspondence.
(271) Unpublished work by the author.
(272) Viguier, P.: Compt. rend. **153**, 955 (1911).
(273) Vilsmeier, A., and Haack, A.: Ber. **60**, 119 (1927).
(274) Vollmann, H., *et al.*: Ann. **531**, 1 (1937).
(275) Volynkin, N.: J. Applied Chem. (U. S. S. R.) **11**, 423 (1938).
(276) Wacek, A., and Kratzl, K.: Cellulosechem. **20**, 108 (1942).
(277) Walker, J. W.: J. Chem. Soc. **69**, 1278 (1896).
(278) Walter, J.: German patent 118,567 (1901).
(279) Wegscheider, R., and Bondi, E.: Monatsh. **26**, 1039 (1905).
(280) Weil, H.: Ber. **41**, 4147 (1908); **44**, 3058 (1911).
(281) Weil, H., and Ostermeier, H.: Ber. **54**, 3217 (1921).
(282) Weil, H., Traun, M., and Marcel, S.: Ber. **55**, 2664 (1922).
(283) Weiss, R., and Beller, A.: Monatsh. **61**, 143 (1932).
(284) Weiss, R., and Koltes, J.: Monatsh. **65**, 351 (1935).
(285) Wenzel, F., and Bellak, L.: Monatsh. **35**, 965 (1914).
(286) West, T.: J. Soc. Chem. Ind. **59**, 275 (1940).
(287) Wieland, H., and Dorrer, E.: Ber. **63**, 404 (1930).
(288) Williams, J. W.: J. Am. Chem. Soc. **61**, 2248 (1939).
(289) Williams, J. W.: Org. Syntheses **23**, 63 (1943).
(290) Wislicenus, H.: J. prakt. Chem. **54**, 61 (1896).
(291) Wittig, G.: Angew. Chem. **53**, 241 (1940).
(292) Wittig, G., Pockels, U., and Droge, H.: Ber. **71**, 1903 (1938); **72**, 89, 884 (1939). Gilman, H., *et al.*: J. Am. Chem. Soc. **61**, 106 (1939); **55**, 1252 (1933).
(293) Wolf, H.: Ber. **61**, 1765 (1928).
(294) Wuyts, H.: Bull soc. chim. Belg. **38**, 195 (1929); **39**, 58 (1930).
(295) Wuyts, H., Berman, L., and Lacourt, A.: Bull. soc. chim. Belg. **40**, 665 (1931).
(296) Wuyts, H., and Koeck, H.: Bull. soc. chim. Belg. **41**, 196 (1932).
(297) Zelinsky, N.: Chem.-Ztg. **28**, 303 (1904).
(298) Zetzsche, F., and Arnd, O.: Helv. Chim. Acta **8**, 591 (1925).
(299) Zetzsche, F., and Arnd, O.: Helv. Chim. Acta **9**, 173, 177 (1926).
(300) Ziegler, K., and Tiemann, P.: Ber. **55**, 3406 (1922).

METABOLITE ANTAGONISTS

RICHARD O. ROBLIN, Jr.

Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut

Received December 17, 1945

CONTENTS

I. Introduction ... 255
II. Historical background ... 256
  A. Enzymology ... 256
  B. Pharmacology ... 260
  C. Chemotherapy ... 261
III. Specific metabolite antagonists ... 263
  A. Amino acids ... 264
  B. *p*-Aminobenzoic acid (PABA) ... 270
  C. Biotin ... 285
  D. Glutathione and other mercapto compounds ... 288
  E. Histamine ... 295
  F. Nicotinic acid; cozymase ... 307
  G. Pantothenic acid ... 310
  H. Purines and pyrimidines ... 322
  I. Riboflavin ... 324
  J. Thiamine; cocarboxylase ... 325
  K. Thyroxine ... 332
  L. Vitamin K; prothrombin ... 342
  M. Miscellaneous antagonists ... 346
IV. General considerations ... 353
  A. Types of antagonism ... 354
  B. Mechanisms of action ... 356
  C. Applications and conclusions ... 359
V. References ... 367

I. INTRODUCTION

The concept that substances chemically related to a metabolite may interfere with the normal function of that metabolite in living cells is attracting widespread interest among chemists and biologists. For the organic chemist, this concept frequently provides a unique starting point for the synthesis of biologically active compounds and the study of the relation between chemical structure and activity. The stimulus for many of the investigations under review came from the discovery of the striking relationship between *p*-aminobenzoic acid and sulfanilamide-type compounds. Not all important antagonists are structurally related to the affected metabolite, however, and the value of specific antagonisms in elucidating the function of metabolites and explaining the action of drugs has been recognized for some time.

It is the object of this review to summarize the pertinent background in several fields of investigation, and to review the available literature[1] relating to the direct antagonism of a number of essential metabolites in living cells. Structural relationships have been emphasized whenever they appeared to be significant. Since the normal function of a metabolite is helpful in accounting for the effects of an antagonist, this aspect of the subject has been reviewed briefly wherever possible.

---

[1] Because of current conditions complete coverage, particularly of the foreign literature, has not been practicable. I should greatly appreciate receiving references to papers which have been overlooked or were not available at the time this review was written.
R. O. R., Jr.

Broadly, a metabolite may be regarded as any substance involved in the chemical processes by which living cells are produced and maintained. But the present review is limited largely to substances of known structure which function as essential parts of cell catalysts (e.g., vitamins and hormones). Since these substances are usually present in minute amounts, only small quantities of an antagonist are required to produce distinctive effects.

Many types of antagonisms are also known. They may be divided roughly into direct chemical or physicochemical effects which frequently obey the law of mass action, and indirect or physiological antagonisms due to opposite but independent actions. Although there is no sharp line of demarcation, in this review the emphasis has been placed on antagonists having a direct, reversible effect on the synthesis or utilization of specific metabolites. In general, naturally occurring antagonisms (i.e., the antagonist is also a metabolite taking part in normal cell processes) and the numerous antagonisms between pairs of drugs have not been included.

II. HISTORICAL BACKGROUND

The current developments in metabolite antagonists can be understood most readily when viewed in the light of previous studies in several fields of investigation, particularly enzymology, pharmacology, and chemotherapy.

A. Enzymology

The use of inhibitors[2] and poisons has long been recognized as an important tool in the study of isolated enzyme systems. Although it is not within the scope of this review, the vast literature in this field forms a rich background for the observations on living cells. Present theories of the nature and specificity of enzymes are, to a considerable extent, founded on the effects of inhibitors. According to these theories, enzymes contain active centers or groups which combine with the substrates. The active centers are presumed to be oriented so as to confer a high degree of specificity.

Tammann (407) in 1891 and Armstrong (13) were among the first to observe that the addition of the products of an enzyme reaction produced an inhibitory effect on its velocity. In many instances, these results cannot be attributed to a mass action effect, but appear to involve a competitive inhibition. Similarly, Haldane (153) and others have called attention to the numerous cases of inhibition of enzymes by substances structurally related to their substrates. These effects are ascribed to a blocking of the active centers by the inhibitors. Haldane

---

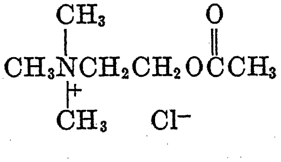
[2] The terminology in different fields is sometimes inconsistent. "Inhibitor" is synonymous with "antagonist" in the discussion of isolated enzymes to conform to usage; elsewhere the term "antagonist" is preferred, to eliminate the situation in which an inhibitor inhibits an inhibitor. To avoid the confusion of ideas arising from a metabolite antagonizing the action of a drug, the drug is referred to as the antagonist and terms such as "preventing" or "reversing" are substituted to describe the effect of the metabolite.

(153) also listed a number of enzyme poisons most of which owed their activity to either a chemical reaction with a reactive group, or salt formation with acidic or basic groups of the enzyme. The plausibility of this explanation was illustrated by Myrbäck (305), who showed that sucrose, glucose, and fructose protected yeast saccharase from inactivation by nitrous acid. This result suggested the presence of an amino group in the active center of the enzyme, the amino group being protected from the nitrous acid by its combination with the substrate or the products of the reaction. Other substances such as maltose and lactose, which did not appear to combine with the enzyme, failed to protect it (153).

In general, inhibitors may affect the velocity of enzymatic reactions in two ways, resulting in either competitive or non-competitive inhibition. Those of the latter type reduce the velocity of the reaction, but the dissociation constant of the enzyme–substrate complex is unaltered. In other words, varying the concentration of substrate has little effect on the degree of inhibition. By contrast, competitive inhibition involves a direct relationship between inhibitor and substrate, so that increasing the concentration of substrate reduces the degree of inhibition, i.e., inhibitor and substrate appear to compete for the same active centers.

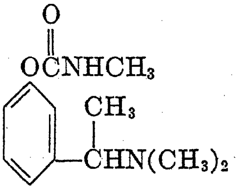
Because of the probability that the fundamental processes in the inhibition of isolated enzymes and the antagonism of metabolites in living cells are related, an examination of some of the more pertinent features of enzyme inhibitors reveals many interesting analogies. A few examples are listed in table 1, but further discussion has been deferred to a later section (*cf.* Section IV).

It is sometimes possible to account for the pharmacological action of drugs in terms of their effect on specific enzyme systems. Thus, Englehardt and Loewi (107) demonstrated that the inhibition of choline esterase by physostigmine resulted in a decreased rate of destruction of acetylcholine. The pharmacological actions of physostigmine are due entirely to its preservation of acetylcholine, the drug itself having no cholinergic activity (69). The Stedmans (399) showed

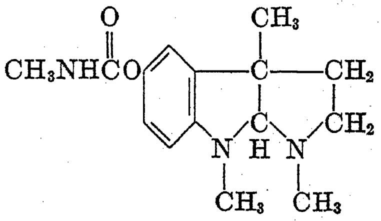


$CH_3\overset{CH_3}{\underset{CH_3}{\overset{+}{N}}}CH_2CH_2OC(=O)CH_3\ Cl^-$

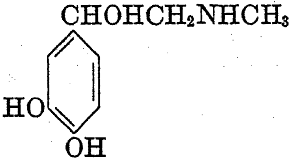
Acetylcholine



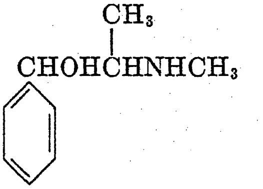
Miotine



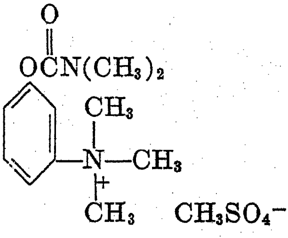
Physostigmine



Epinephrine



Ephedrine



Prostigmine methylsulfate

TABLE 1

Inhibitors of isolated enzyme systems

ENZYME	SUBSTRATE	INHIBITOR	OTHER OBSERVATIONS	REFERENCE
Choline esterase	Acetylcholine	Physostigmine (eserine)	Competitive inhibition; accounts for pharmacological activity of physostigmine (see text)	Loewi and Navratil (242)
Liver esterase	Tributyrin	Octyl alcohol	1.6×10^{-5} *M* stimulates, but 2.5×10^{-4} *M* or greater causes increasing inhibition	Sobotka and Glick (387)
Liver esterase	Tributyrin	Sodium arsanilate	Much less susceptible to inhibition by quinine than serum esterase	Rona and Pavlovic (349)
Serum esterase	Tributyrin	Quinine	Much less susceptible to inhibition by sodium arsanilate than liver esterase	Rona and Pavlovic (349)
Pancreatic lipase	Ethyl butyrate	Acetophenone	Competitive inhibition; other ketones also inhibitory but not the oxime of acetophenone	Murray (304)
Malt amylase	Starch (maltose production)	Glucose; maltose	Fructose and sucrose not inhibitory	Wohl and Glimm (439)
Yeast saccharase	Sucrose	β-Glucose; fructose	Competitive inhibition; α-glucose not inhibitory	Kuhn (204)
Aspergillus saccharase	Sucrose	α-Glucose	Competitive inhibition; β-glucose and fructose not inhibitory	Kuhn (204)
Arginase	Arginine	Ornithine	Urea not inhibitory, indicating that action of ornithine is not due to displacement of mass action equilibrium	Gross (150)

Histidase	*l*-Histidine	*d*-Histidine	Imidazole, guanidine, arcaine, and ethylenediamine also inhibitory, but guanidoacetic acid inactive	Edlbacher, Baur, and Becker (99)
Intestinal peptidases	Glycylglycine	Glycine; alanine	Competitive inhibition; glycine anhydride inhibitory but not benzoyl glycylglycine, which is also not hydrolyzed by enzyme	Euler and Josephson (114)
Intestinal peptidases	Glycyl-*m*-aminobenzoic acid	Glycyl-*o*-aminobenzoic acid	Glycyl-*p*-aminobenzoic acid also hydrolyzed by enzyme and inhibited by ortho isomer	Balls and Kohler (23)
Lactic dehydrogenase	Lactic acid	Pyruvic acid	Competitive inhibition; also produced by other substances with acidic groupings —CO—COH* or —CHOH—COH* (H* is labile)	Quastel and Wooldridge (328)
Succinic dehydrogenase	Succinic acid	Malonic acid	Competitive inhibition; other substances with —C—CH—COOH or —C—CH_2—COOH configuration also inhibitory	Quastel and Wooldridge (328)
Xanthine oxidase	Xanthine	3-Methylxanthine	Guanine, uric acid, 1(or 7)-methylguanine also strong inhibitors	Coombs (76)
Monamine oxidase	Epinephrine	Ephedrine	Competitive inhibition; accounts for pharmacological activity of ephedrine (see text)	Blaschko, Richter, and Schlossmann (40)
Diamine oxidase (histaminase)	Histamine	Guanidine	Competitive inhibition; *N*-methylation increases inhibiory action	Zeller (470)

that synthetic methyl urethans related to physostigmine, such as miotin, also inhibit esterases. They suggested that the inhibition could be explained by the ester-like structure of the urethans, which enables them to combine with and block the active centers of the esterases. Miotine and prostigmine (5) closely resemble physostigimine in their pharmacological effects. In an analogous fashion Gaddum and Kwiatkowski (138) considered that the pharmacological action of ephedrine is due to its sparing effect on the enzymatic destruction of epinephrine (table 1). Quastel and coworkers (326, 327) studied the effects of anesthetics and hypnotics on the enzymes of the brain. The action of many of these substances is attributed to the inhibition of a sensitive component of the brain respiratory system. Because of the intricate balance of many enzyme systems, antagonisms in living cells are obviously much more complex than the inhibition of isolated enzymes. A comprehensive review of the relation of drugs to the inhibition of specific enzymes has been written by Bernheim (34).

B. Pharmacology

Physiologists and pharmacologists for many years have employed antagonists to study the normal functions of metabolites and to determine the mode of action of drugs. An excellent survey of these investigations has been made by Clark (69), who classified the three chief forms of drug antagonism as follows:

> "(a) *Chemical antagonism in vitro.* In this case the antagonists react together to form a product of reduced activity.
>
> "(b) *Physiological antagonism.* In this case two drugs produce opposite but independent effects on cells or organs.
>
> "(c) *Specific antagonism.* This term is reserved for cases in which one drug inhibits the action of another drug on living cells, although no reaction between the drugs occurs in vitro."

In this classification Clark was considering antagonisms between pairs of drugs (e.g., pilocarpine and atropine) as well as metabolite antagonists. Presumably type a antagonisms can occur *in vivo* as well as *in vitro*, and the action of arsenicals may fall in this category (*cf.* Section III,D). Physiological antagonisms (type b) have not been considered in this review, since no direct relationship between metabolite and antagonist is involved.

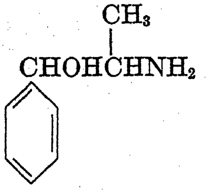
One of the simplest systems of type c is the antagonism by carbon monoxide of the combination of oxygen and hemoglobin, i.e., $CO + HbO_2 \leftrightarrows O_2 + HbCO$. This system was investigated from a quantitative standpoint as early as 1884 by Hüfner (180) and later by Douglas, Haldane, and Haldane (93) and Hartridge and Roughton (164). Both oxygen and carbon monoxide form reversible compounds with hemoglobin and appear to compete for the same active centers or receptors. In dilute aqueous solution the distribution of hemoglobin between the two gases is given by the following formula:

$$\frac{[O_2]}{[CO]} \times \frac{[HbCO]}{[HbO_2]} = K$$

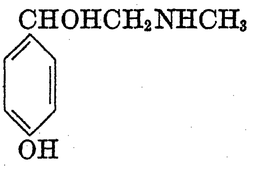
The concentration of HbCO equals that of HbO_2 when the relative pressures of oxygen and carbon monoxide are approximately 200 to 1.

Külz (211) and Raventos (333) studied the action of a series of tetraalkylammonium salts on isolated frog muscle (rectus abdominis). They found that in the series $(CH_3)_4NX$ to $(CH_3)_3N(C_4H_9)X$ the salts, like acetylcholine, produced contractions. As the chain length increased, the effect decreased, so that $(CH_3)_3N(C_8H_{17})X$ not only failed to produce contractions, but in fact exerted a powerful antagonistic effect on the action of the lower members of the series. Both $(CH_3)_3N(C_8H_{17})X$ and $(C_2H_5)_4NX$ antagonized the action of acetylcholine and of the $(CH_3)N(R)X$ type compounds on the frog's heart. In other tissues there were wide variations in the types of action and antagonism.

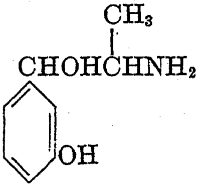
Modern and Thienes (297) investigated the antagonistic effects of a large group of compounds structurally related to epinephrine. They found that under certain conditions compounds such as propadrine and *d*-synephrine antagonized the action of epinephrine on smooth muscle, whereas *d*-metasynephrine was inactive.



Propadrine



d-Synephrine



d-Metasynephrine

The antagonists by themselves frequently had no effect on the motility of the muscle.

In 1937, Clark (69) pointed out that such results suggested the general hypothesis that substances are most likely to be antagonized by compounds of similar molecular configuration.

Quantitative studies of the acetylcholine–atropine (67) and epinephrine–ergotoxine (135, 287) antagonisms, in which the ratio of metabolite to antagonist remains constant over a wide range of concentrations, indicate a competition for the same tissue receptors (68, 69, 136). On this basis it is assumed that the union of drugs with specific receptors involves two processes: (*a*) fixation of drug, and (*b*) selective action after fixation. An antagonist either prevents fixation, or alters the pattern of receptors so that the active drug or metabolite can no longer produce its specific effect (69).

C. Chemotherapy

From the time when he founded modern chemotherapy, Ehrlich emphasized the importance of attempting to determine the mechanism of action of chemotherapeutic agents. Drawing upon his immunological studies and observations with drug-resistant trypanosomes, he developed the famous chemoreceptor or side-chain theory. This theory postulated the presence of chemical groups or side chains on the organisms with which the chemotherapeutic agents combined. The side chains were considered to be essential for the nutrition of the trypanosomes. Present concepts of the nature and structure of protoplasm have required major modifications in Ehrlich's original ideas, but the fundamental principle of interference with metabolic processes remains as a tribute to his brilliant imagination. Voegtlin (422) pointed out that if the —SH group is regarded as the arsenic receptor, one finds a basis for Ehrlich's interpretation of the mode of action of arsenicals (*cf.* Section III,D). The observations of Yorke and coworkers (467) and Hawking (166), indicating that vulnerable trypanosomes concentrate arsenicals whereas resistant strains do not, has since helped to explain the differences in the susceptibility of trypanosomes and in their toxicity to host cells.

In the study of germicides several pioneer investigators attempted to relate the action of various agents to their effect on bacterial metabolism. While much of the early work was concerned with bactericidal action, Simon and Wood (378) in 1914 examined the mechanism of action of a group of aniline dyes which were primarily bacteriostatic. Like Ehrlich, these investigators believed that the growth-inhibitory action was probably due to a combination of the dyes with "nutriceptors" in the organism, the dye becoming anchored so that the organisms starved or were unable to multiply. Dye-resistant strains were developed, and this phenomenon was considered to be due to the formation of receptors which either did not attract the dye or were able to destroy it. Other investigators (49, 181, 401) carried these ideas as far as the limited contemporary knowledge of bacterial metabolism would permit. It was not until developments in other fields (e.g., biochemistry, enzymology, and nutrition) had filled in the background that a more complete picture could be drawn.

The theory that the bacteriostatic action of sulfanilamide-type compounds is due to the blocking of an essential enzyme system or systems was adumbrated by several investigators, notably Lockwood (237), McIntosh and Whitby (267), Stamp (393), and Green (148). It remained for Woods and Fildes (122, 443, 446) to provide a firm foundation for this theory and to extend it to the broad basis which has inspired so many subsequent investigations of other bacterial metabolite antagonists.

In 1940 Woods (443) isolated in a crude form from yeast cells a substance which prevented the antibacterial action of sulfanilamide and sulfapyridine. Stamp (393) and Green (148) had obtained similar extracts from other organisms. After concentrating his factor, Woods concluded from an examination of its chemical properties that the substance might be structurally related to sulfanilamide. The behavior of the material, resembling the competitive inhibition of enzyme reactions by compounds structurally related to the substrate or product, pointed to the same conclusion. Accordingly, *p*-aminobenzoic acid (PABA) was tried and found to be highly active. In order to reverse or prevent the bacteriostatic effect of sulfanilamide, a more or less constant molar ratio of PABA to sulfanilamide was required over a considerable range of concentrations. Both the unsubstituted amino group and the carboxyl group para to it proved to be necessary for antisulfanilamide activity. Woods considered that the results provided strong circumstantial evidence for the identity of the yeast factor, and suggested that PABA is an essential metabolite for the growth of microörganisms.

At the same time, Fildes (122) expanded the concept of metabolite antagonists in relation to bacterial growth in an illuminating paper entitled, "A Rational Approach to Research in Chemotherapy". He defined essential metabolites as the substances involved at "each stage in any synthesis necessary for growth, without which, either synthesized or supplied from outside, growth cannot occur." A growth factor for a given organism is then an essential metabolite which it cannot synthesize. For example, *E. coli*, which grows on a medium containing only inorganic salts and glucose, can synthesize all its other requirements, whereas *P. vulgaris* needs preformed nicotinic acid. Given nicotinic acid, *P. vulgaris* can complete the synthesis of cozymase, which is also produced by *E. coli*. Nicotinic acid is regarded as a growth factor for *P. vulgaris* and an essential metabolite for *E. coli*, and cozymase an essential metabolite for both organisms. Fildes proposed the theory that antibacterial substances may inhibit growth by antagonizing or interfering with an essential metabolite in one of three ways:

"1. By oxidizing a substance which requires to be reduced.

"2. By molecular combination giving an inactive product.

"3. By competition for an enzyme associated with the essential metabolite."

Various oxidizing agents such as ferricyanide or methylene blue were presumed to act by the first mechanism, while the combination of mercuric chloride with essential —SH groups was regarded as an example of class 2. Fildes and Woods considered that, because of their structural similarity, sulfanilamide and PABA compete for an enzyme involved in the further utilization of PABA. On this basis, the sensitivity of an organism to sulfanilamide was considered to depend in part on its ability to synthesize PABA. More broadly, Fildes suggested that compounds closely related structurally to other known metabolites, but devoid of essential metabolic activity, might also be found to inhibit the growth of various microörganisms. Experimental verification for many of the ideas proposed by Fildes and Woods will be found in the subsequent sections of this review.

III. SPECIFIC METABOLITE ANTAGONISTS

The following sections, arranged by metabolites, cover most of those for which any extensive literature has accumulated. Other recognized metabolites which might have been included, such as acetylcholine and epinephrine, are described briefly in Section II. All but the recent literature relating to antagonists of these two metabolites has been discussed in detail by Clark (69). Some of the later studies in these fields are incorporated in Section IV. A number of articles summarizing a portion of the work with other metabolite antagonists, particularly in relation to antibacterial action, have appeared (6, 7, 94, 199, 205, 256, 257, 261, 425, 428, 451). The reader is referred to these summaries for brief discussions of this phase of the subject.

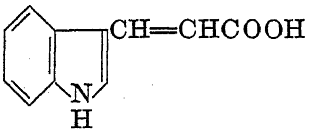
An attempt has been made to standardize terminology[2], so that the term "inhibition ratio" is used throughout to indicate the moles of antagonist required to nullify the function of one mole of metabolite. In certain instances, the ratio may be reported on the basis of a 50 per cent reduction, in which case these conditions are mentioned specifically. It is important to distinguish between these two criteria in making comparisons, because the ratio required for complete antagonism is frequently many times greater than at the 50 per cent level, and this relationship may vary with different antagonists or with the same antagonist in different cells.

The criterion of antagonism has been based primarily on the reversal or prevention of the biological effect of an antagonist by a specific metabolite, recognizing that this test can only be regarded as an important but not exclusive condition for the evaluation of direct antagonists. Other limitations of scope are outlined in Section I. The papers in each section have been reviewed in more or less chronological order, with some exceptions for the sake of continuity.

A. Amino acids

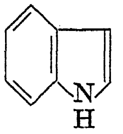
Many organisms are unable to synthesize one or more of the known amino acids. These essential metabolites are the building blocks of the cell proteins, and as such they are usually required in larger quantities than many of the other growth factors. Proteins are not only the basic units of cell structure but also, in conjunction with active prosthetic groups, assume the rôle of enzymes to catalyze many metabolic reactions (296). Finally, some of the amino acids probably serve as precursors of other essential metabolites (88).

In studying the production of indole from tryptophan, Fildes (121) noted that the growth of *E. coli* was inhibited by indoleacrylic acid. Following up this earlier observation, he found (124) that $M/8000$ I prevented growth, while a number of related substances, such as indoleacetic and propionic acids, produced very slight or no growth inhibition. Since indoleacrylic acid is closely related to II in several respects, Fildes reasoned that I might interfere with an enzyme system concerned with the metabolism of tryptophan. He demonstrated experimentally that, although not itself a growth stimulant, II counteracted the inhibition produced by I. The growth was proportional to the amount of tryptophan added, but there was no quantitative relation between the concentrations of I and II. Unlike the sulfonamide–PABA relationship, the response of the organisms was a function of the tryptophan concentration only, regardless of the amount of indoleacrylic acid present. These results, which were confirmed with *B. typhosum*, led to the suggestion that I interferes with the formation of tryptophan rather than its utilization. Under these conditions no quantitative relationship between I and II would be expected, and the minimum amount of tryptophan necessary for growth should reverse any reasonable amount of indoleacrylic acid. However, there should be a quantitative relationship between I and a precursor of tryptophan. Indole is a precursor for *B. typhosum*, but no interference with the action of I by this substance was found. To explain the lack of any reversing action with indole, Fildes assumed that either the blocking effect of I on tryptophan synthesis is not reversible or, because of the growth inhibition produced by indole itself at higher concentration, the effect could not be demonstrated.

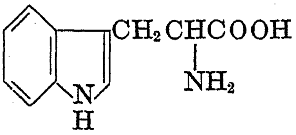


I

β-Indoleacrylic acid



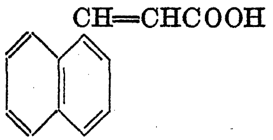
Indole



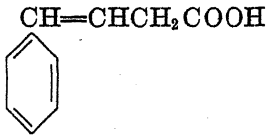
II

Tryptophan

Block and Erlenmeyer (42) reported that l-naphthylacrylic acid and styrylacetic acid resembled indoleacrylic acid in their behavior. These substances ap-



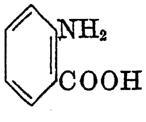
1-Naphthylacrylic acid



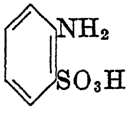
Styrylacetic acid

peared to antagonize the growth-stimulating action[3] of tryptophan. While *trans*-cinnamic acid gave similar results, the authors concluded that tryptophan metabolism was not involved in this case. Dihydrocinnamic, benzoic, and fumaric acids produced no effect.

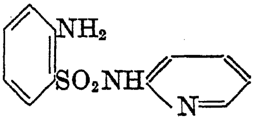
With several species of lactic acid bacteria, Snell (381) was able to substitute anthranilic acid for tryptophan. Since anthranilic acid is an isomer of *p*-aminobenzoic acid (PABA), he studied the corresponding isomer of sulfanilamide (orthanilamide) as well as orthanilic acid and 2-(orthanilamido)pyridine. None of these substances inhibited growth promoted by anthranilic acid. Snell concluded that, if it existed at all, the antibacterial power of orthanilamide in relation to anthranilic acid was of a much lower order of magnitude than in the case of the corresponding sulfanilamide–PABA relationship.



Anthranilic acid

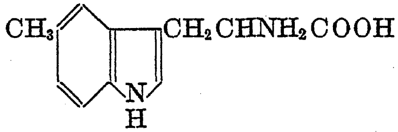


Orthanilic acid



2-(Orthanilamido)pyridine

Anderson (12) found that the bacteriostatic action of *dl*-5-methyltryptophan on *E. coli* in simple media was reversed by *l*-tryptophan, the inhibition ratio being approximately 1000:1 over a considerable range of concentrations. On the other hand, he also demonstrated that the tryptophan requirements of certain *E. coli* bacteriophages could be replaced by the 5-methyl derivative. Earlier, Gordon and Jackson (145) had shown that the latter compound was toxic to rats on a tryptophan-deficient diet, but had no effect on animals receiving an adequate ration.

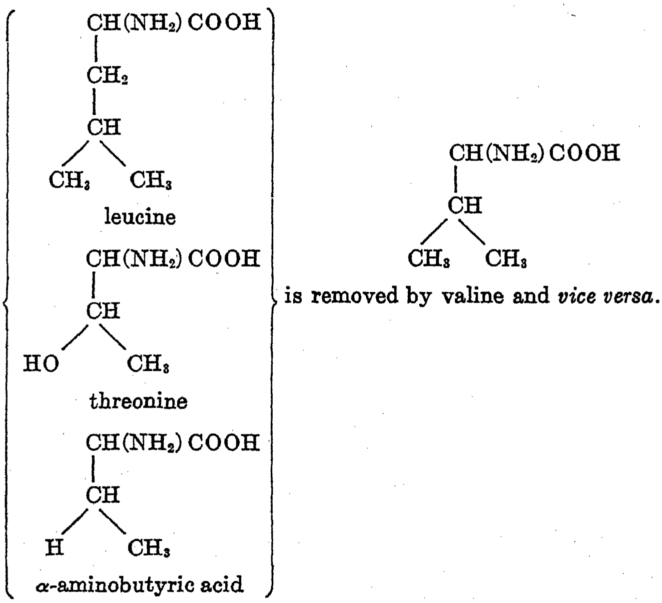


5-Methyltryptophan

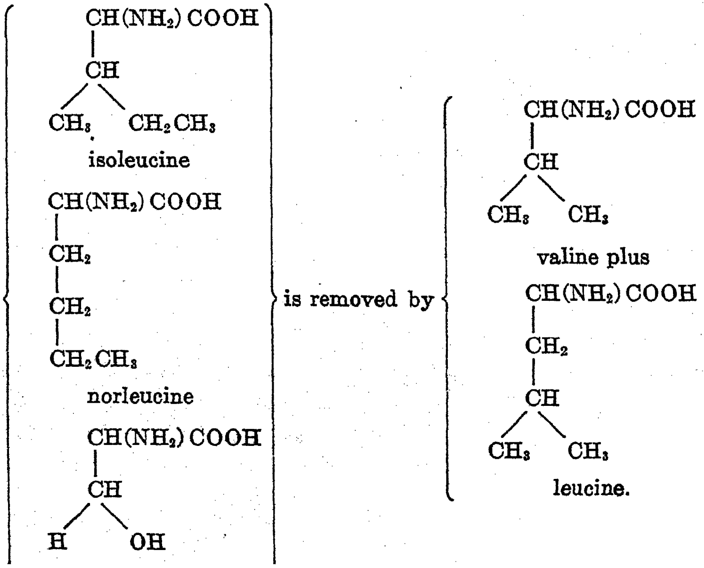
Gladstone (141), investigating the amino acid requirements of *B. anthracis*, observed a number of curious interrelationships among groups of chemically related amino acids. When leucine or valine was eliminated from the synthetic

[3] Growth stimulation usually indicates that the metabolite is a limiting factor for the growth of the particular organism, rather than a growth factor as defined by Fildes (122). Thus, the synthetic ability of the organism for the metabolite is limited, and addition of the preformed metabolite provides a more favorable medium for growth. Under these conditions, any growth inhibitor is likely to be less effective, regardless of whether it is a specific antagonist for the metabolite in question. Consequently, specific antagonisms are frequently more difficult to establish when the metabolite is a growth stimulant.

medium, the organisms failed to grow, and the removal of isoleucine resulted in delayed and incomplete growth. The absence of all three amino acids, surprisingly enough, allowed growth to occur, but the addition of any one of these amino acids to a mixture in which all three were originally absent completely inhibited growth. Isoleucine was the most effective, preventing growth at a concentration of $M/312{,}500$ under these conditions. The presence of the unnatural isomers did not account for the results, since the same effects were obtained with the purified natural isomers. Further study revealed several other similar relationships, which were summarized as follows by Gladstone:

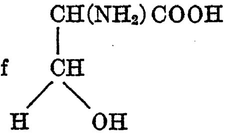

The toxicity of

is removed by valine and *vice versa*.


The toxicity of

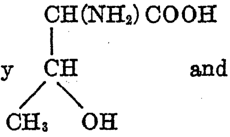
is removed by

The toxicity of



serine

is removed by



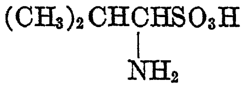
threonine

and *vice versa*.

On the basis of the similarity in chemical structure of the growth inhibitors and their antagonists, Gladstone (141) suggested that some common reaction necessary for growth might be involved. Thus, an excess of one amino acid might block the enzymes necessary either for the synthesis of another chemically related, or for building it, when synthesized, into more complex substances.

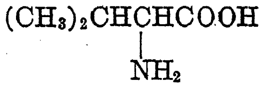
Numerous other instances in which one amino acid may interfere with the utilization of another have been reported. The results of these studies are summarized in table 2. It is usually assumed in these cases that the antagonist is also an essential metabolite. However, an excess of one (the antagonist) blocks the function of the other (the metabolite), because of the structural similarity between them. Such effects are most frequently observed in relatively simple culture media. Results of this type do not come strictly within the scope of this review, but they have been included in this instance because of the numerous interesting structural relationships involved.

Several α-aminosulfonic acids structurally related to the natural α-aminocarboxylic acids were studied by McIlwain (251, 252). The sulfonic acid analogs of amino acids such as glycine, alanine, leucine, and valine were prepared by treating the corresponding aldehyde bisulfite derivatives with aqueous ammonia (251). It was found (252) that in a chemically defined medium organisms which required preformed amino acids were inhibited by the α-aminosulfonic acids. There appeared to be considerable overlapping in the reversing effect of the amino acids. Thus, the growth inhibition produced by α-aminoisobutanesulfonic acid was partly reversed by glycine or alanine, more effectively by valine, but not at all by leucine. However, of the various metabolites tested, only the amino acids were capable of preventing the growth inhibition of the α-aminosulfonic acids. In general, bacteria synthesizing their own amino acids were not susceptible. McIlwain also investigated the action of cysteic acid, taurine and tauramide, the respective analogs of aspartic acid and β-alanine. These sulfonic acids were not inhibitory, and sometimes stimulated suboptimal growth.



$$(CH_3)_2CH\underset{\displaystyle NH_2}{\underset{|}{C}}HSO_3H$$

α-Aminoisobutanesulfonic acid



$$(CH_3)_2CH\underset{\displaystyle NH_2}{\underset{|}{C}}HCOOH$$

Valine

Spizizen (391), investigating the multiplication of bacteriophage acting on *E. coli*, found that the reproduction of this bacterial virus could be stimulated by glycine or glycine anhydride. He demonstrated that the multiplication of phage stimulated in this manner could be inhibited by aminomethanesulfonic acid, the sulfonic acid analog of glycine. On the contrary, when xanthine was used as a stimulant, no inhibition was observed.

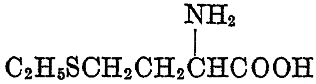
Antagonistic relationships between amino acids

ANTAGONIST	METABOLITE	ORGANISM	OTHER OBSERVATIONS	REFERENCE
$H_2NCH_2CH_2COOH$ β-Alanine	$H_2NCOCH_2CH(NH_2)COOH$ Asparagine	Yeast	β-Alanine acts as growth stimulant only in presence of asparagine or aspartic acid	Nielsen and Hartelius (307)
$CH_3CH_2CH_2CH_2CH(NH_2)COOH$ Norleucine	$CH_3SCH_2CH_2CH(NH_2)COOH$ Methionine	*Escherichia coli*	Norvaline also antagonistic; inhibition ratio norleucine: methionine approximately 1000:1	Harris and Kohn (159)
$HOOCCH_2CH(NH_2)COOH$ Aspartic acid	$H_2NCOCH_2CH_2CH(NH_2)COOH$ Glutamine	*Lactobacillus casei*	Asparagine and glutamic acid also effective in preventing growth inhibition caused by aspartic acid	Feeney and Strong (117)
$CH_2(NH_2)COOH$ Glycine	$CH_3CH(NH_2)COOH$ α-Alanine	*Streptococcus fecalis* R	Bacteriostatic action of glycine also reversed by pyridoxine	Snell and Guirard (384)
$(CH_3)_2CHCH_2CH(NH_2)COOH$ Leucine	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$ Isoleucine	*Pasteurella pestis*	Valine also reverses antagonistic action of leucine; not due to growth-promoting action	Doudoroff (92)

Phenylalanine *or* norleucine *or* norvaline....	Isoleucine *and* valine	*Neurospora crassa*	Antagonistic effect limited to mutant strain requiring preformed isoleucine and valine	Bonner, Tatum, and Beadle (43)
$H_2NC(=NH)NH(CH_2)_3CH(NH_2)COOH$.... Arginine	$H_2NCH_2(CH_2)_3CH(NH_2)COOH$ Lysine	*Neurospora crassa*	Only natural forms of amino acids showed effects—limited to mutant strain; inhibition ratio approximately 2:1	Doermann (89)
Norleucine..............................	Methionine	*Proteus morganii*	Norvaline and allothreonine also antagonistic but less specific; ratio norleucine: methionine 1000:1 (*cf.* Harris and Kohn (159))	Porter and Meyers (325)
Asparagine..............................	β-Alanine*	Yeast	Growth stimulated by pantothenic acid unaffected by asparagine (*cf.* Nielsen and Hartelius (307))	Sarett and Cheldelin (361)

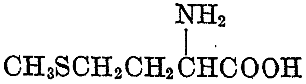
* Other β-alanine antagonists are included under pantothenic acid.

The preparation of ethionine was described by Dyer (96), who showed that it was toxic for rats on a methionine-deficient diet. She also demonstrated that the toxicity could be overcome by additional methionine. In the course of their studies on sulfonamide inhibitors, Harris and Kohn (159) observed that ethionine inhibited the growth of *E. coli* on a synthetic medium. *M*/1000 ethionine produced a 50 per cent inhibition of growth, which was completely reversed by *M*/10,000 methionine.



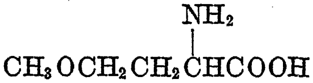
$C_2H_5SCH_2CH_2\overset{\overset{NH_2}{|}}{C}HCOOH$

Ethionine



$CH_3SCH_2CH_2\overset{\overset{NH_2}{|}}{C}HCOOH$

Methionine

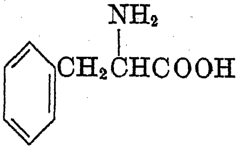


$CH_3OCH_2CH_2\overset{\overset{NH_2}{|}}{C}HCOOH$

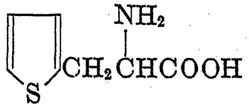
Methoxinine

Roblin, Lampen, English, Cole, and Vaughan (341) synthesized methoxinine and studied its action on *E. coli* and *Staph. aureus*. The growth-inhibitory effect of this compound was prevented by *l*-methionine but not by the *d*-isomer. One mole of *dl*-methionine reversed the antibacterial action of 500 to 1000 moles of *dl*-methoxinine or *dl*-ethionine. In combination with sulfonamides a synergistic bacteriostatic effect was produced by methoxinine. Similar results were obtained with ethionine, confirming the observations of Harris and Kohn (159). Other investigations involving a relationship between methionine and the sulfonamides are incorporated in the section on PABA antagonists.

An investigation of thienylalanine (469) was carried out by du Vigneaud, McKennis, Simmonds, Dittmer, and Brown (420). They found that the growth of a strain of yeast, *S. cerevisiae*, was inhibited by thienylalanine. Although growth inhibition was not complete, phenylalanine reversed the maximum effect in a ratio of approximately 1:2. Other amino acids, including tyrosine, were ineffective in preventing the growth-inhibitory action. Preliminary experiments with thienylalanine in rats gave inconclusive results, although some evidence of a phenylalanine antagonism was obtained.



Phenylalanine



β-2-Thienylalanine

B. p-Aminobenzoic acid (PABA)

Because of the unconventional manner in which PABA emerged as a bacterial growth factor, its general acceptance developed slowly. The intensive studies which followed the reports of Woods and Fildes (443, 446; see Section II) have, for the most part, provided strong supporting evidence for the validity of their suggestions concerning PABA and its relation to the mechanism of action of sulfanilamide-type compounds.

PABA or its derivatives have been isolated from yeast (38, 208, 355) and found to have growth factor activity for *Clostridium acetobutylicum* (213, 316, 355, 356) and several other microörganisms (182, 215, 232). Mutant strains of *Neurospora crassa* (409) and *E. coli* (348) requiring preformed PABA have also been obtained. By means of microbiological assays, the presence of the substance in many bacteria (220) and other natural sources has been demonstrated (170, 311), although it has been pointed out that this method of identification is not conclusive (170). Sulfonamide-resistant strains of some organisms have been shown to possess increased ability to synthesize PABA (bioassay) (221), although this is not the only means by which resistance can develop (183).

Much of the uncertainty regarding PABA has arisen from the conflicting reports of the action of related compounds. A number of these substances show considerable antisulfonamide activity (170, 311). Others, such as *p*-aminophenylacetic acid, have been reported to possess high growth factor activity but no reversing effect on sulfonamides (356). This observation has not been confirmed by other investigators (179, 214, 218, 222, 464). With few exceptions only those compounds which are converted to PABA, either by the organisms or spontaneously, can replace it as a growth factor or antisulfonamide[4].

The so-called secondary sulfonamide-reversing agents (201), such as methionine (41, 159, 405) and the purines (160, 203, 280, 385) as well as peptone and other complex substances, represent another factor which has limited the widespread acceptance of the Woods–Fildes theory. However, if PABA represents only one stage in a complex series of metabolic processes, it seems reasonable that supplying precursors or products of subsequent stages may also prevent or limit the growth-inhibiting action of substances antagonizing the utilization of PABA. Moreover, under certain conditions proper supplements may promote the development of alternate routes to the same metabolic end-point and so render microörganisms insensitive to PABA antagonists.

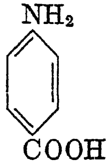
Only some of the most pertinent results are included in this review. A complete and detailed account of the voluminous literature in this field can be found in more extensive reviews and books, such as those of Northey (310, 311) and Henry (170, 171). Since other explanations for the mode of action of sulfonamides are also discussed by these authors, these alternative theories are not included here. Moreover, in the opinion of this reviewer, they fail to explain more than a small fraction of the observations in this field as satisfactorily as the Woods–Fildes theory. It is also evident, however, that no single mechanism of action has yet been devised which will allow all of the multitudinous observations on the sulfonamides to be fitted into prescribed niches.

---

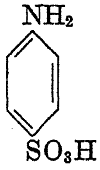
[4] In assessing results with substances which are active in extremely small quantities, the difficulty of avoiding contamination should be recognized. In one of our laboratories in which PABA was in common use, it was practically impossible to prepare a medium free of this factor. Using the same reagents in another laboratory, however, no difficulty was experienced in preparing a PABA-free medium.

1. PABA antagonists: sulfanilamide derivatives

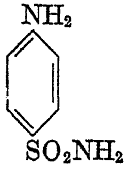
Woods (443), in his original paper, described the more or less constant ratio (5,000–25,000:1 for *Strep. hemolyticus* or *E. coli*) associated with the reversal of the antibacterial activity of sulfanilamide by PABA. This effect was also observed with the yeast extract (*cf.* Section II). He attributed the ratio to a competition between sulfanilamide and PABA for an enzyme system associated with the utilization of PABA as an essential metabolite. Woods also pointed out the close structural relationship between metabolite and antagonist. These considerations led him to examine PABA for antisulfonamide activity. Under the test conditions with *Strep. hemolyticus*, sulfapyridine had five times the antibacterial activity of sulfanilamide. PABA also prevented the action of sulfapyridine, although five times as much was required as for an equimolar concentration of sulfanilamide. The weaker growth-inhibitory action of sulfanilic acid was also reversed by PABA. After testing a number of other substances for antisulfonamide activity, Woods concluded that in general both carboxyl and free amino groups para to one another appeared to be necessary. Acetylation of the amino or esterification of the carboxyl groups of PABA resulted in a thousandfold or greater reduction in the antisulfonamide activity. Similar results were obtained with the yeast factor. Summing up the evidence, Woods presented the following hypothesis of the possible mode of action of sulfanilamide:



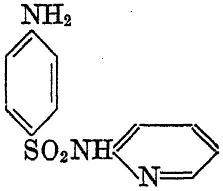
p-Aminobenzoic acid (PABA)



Sulfanilic acid



Sulfanilamide



Sulfapyridine

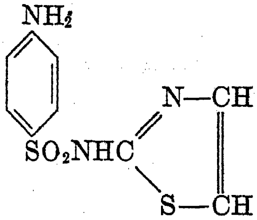
> "In the first place it is suggested that *p*-aminobenzoic acid is essential for the growth of the organism. It is, however, normally synthesized in sufficient quantity by the present strain of streptococcus (and by coli) since it is not necessary to add it to a medium containing only known substances or preparations known to be free from anti-sulfanilamide activity (McIlwain unpublished). It can also be extracted from the streptococcal cell [Stamp (393)]. On the basis of the experimental work it is next suggested that the enzyme reaction involved in the further utilization of *p*-aminobenzoic acid is subject to competitive inhibition by sulfanilamide and that this inhibition is due to a structural relationship between sulfanilamide and *p*-aminobenzoic acid (which is the substrate of the enzyme reaction in question)."

Selbie (368) demonstrated that PABA also prevented the chemotherapeutic action of sulfanilamide in mice infected with streptococci, indicating that the modes of action *in vivo* and *in vitro* are closely related, if not identical. Similar results were obtained by Findlay (126), who also reported that the effect of sulfanilamide on the virus of lymphogranuloma venereum in mice was reversed by PABA. He reasoned that PABA might be considered as an essential metabolite only for those viruses which are susceptible to sulfonamides. Findlay's results with lymphogranuloma were not confirmed by Seeler, Graessle, and Dusenberg (367). However, other investigators (230, 246, 275, 277, 303, 330) corroborated, in general, the *in vivo* reversal of various sulfanilamide derivatives and sulfones by PABA with a variety of infectious agents.

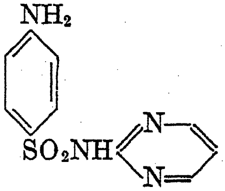
The *in vitro* observations of Woods (443) were confirmed by MacLeod (272), Rubbo and Gillespie (355), and in many subsequent studies. In addition to bacteria, MacLeod (272) found sulfonamide-reversing agents in many body tissues and other natural sources. Some chemical differences between the naturally occurring substances and PABA, such as the extractability with ether below pH 4.5, were observed. MacLeod also showed that a sulfapyridine-fast strain of pneumococcus produced considerably more antisulfonamide than the parent strain.

Rubbo and Gillespie (355) first demonstrated that PABA is a growth factor for *Clostridium acetobutylicum*. These investigators isolated *p*-benzoylaminobenzoic acid from a benzoylated yeast extract. They also observed a close correlation between the growth factor and antisulfonamide activity of a number of related compounds. The molecular inhibition ratio between sulfanilamide and PABA was found to be 23,000:1.

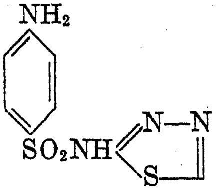
Numerous other investigators (84, 196, 222, 231, 245, 299, 302, 389, 405, 427, 431) extended Woods's observations to additional sulfonamides, sulfoxides, and sulfones, and to many other organisms. Employing pneumococcus type III, Strauss, Lowell, and Finland (405) demonstrated the roughly linear relationship between the concentrations of sulfonamides (sulfanilamide, sulfapyridine, and sulfathiazole) and the minimum concentration of PABA required to prevent their bacteriostatic action (figure 1). They also noted that the concentration of PABA required to reverse the sulfonamides was directly related to their antibacterial activity. Thus, under the conditions of the experiment, approximately



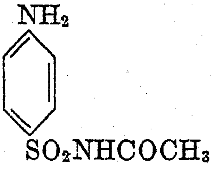
Sulfathiazole



Sulfadiazine



Sulfathiadiazole



Sulfacetimide

ten times as much PABA was required to prevent the action of 10 mg. per 100 cc. of sulfathiazole as was needed for the same concentration of sulfanilamide. Similar results were obtained by Spink and Jermsta (389) with *Staph. aureus* and various sulfonamides (sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine).

A number of sulfonamides, including a series of alkyl thiadiazoles, was investigated by Kimmig (196), who found that in every case their antigonococcal action was prevented by PABA. No antisulfonamide activity could be demonstrated in gonococcal cells. Compounds related to PABA, such as the ortho and meta isomers and *p*-nitroaniline, showed no sulfonamide-reversing action.

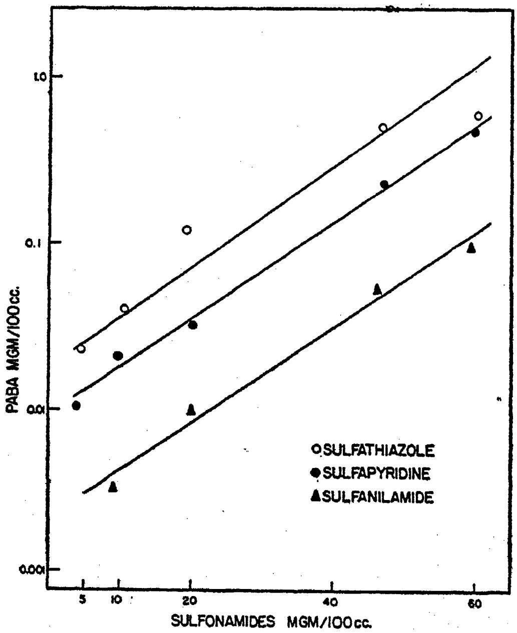
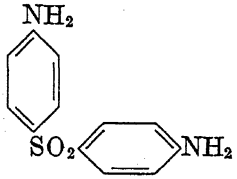


FIG. 1. Minimum concentrations of PABA required to reverse the bacteriostatic action of varying concentrations of three sulfonamides (semi-logarithmic scale). Strauss, Lowell, and Finland (405).

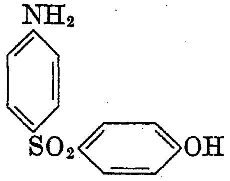
Lwoff, Nitti, Tréfouël, and Hamon (245) studied the action of sulfanilamide on the flagellate *Polytomella caeca.* The primary effect appeared to be on cell division, since the organisms increased to abnormal size but multiplied at a much reduced rate. At pH 4, five times as much sulfanilamide was required to produce the same result as that obtained at pH 7. PABA prevented the action of sulfanilamide, although the metabolite showed an even greater variation with pH. After correction for the change in the activity of sulfanilamide, the inhibition ratio was found to be approximately 1 from pH 9.10 to 7.5. From pH 7.0 to 3.65 the ratio increased to 245, and then declined gradually to 76 at pH 2.25. The maximum ratio corresponded closely with the isoelectric point of PABA. Lwoff and coworkers suggested that these results indicated that the unionized form of the metabolite penetrated the cells much better than the ionized form.

The action of sulfanilamide on *E. coli* and *Aspergillus niger* also varied somewhat with pH, the organisms being less sensitive in acid media. With these organisms, however, the sulfanilamide–PABA ratio (*ca.* 10,000:1 for *E. coli*) changed only slightly with pH.

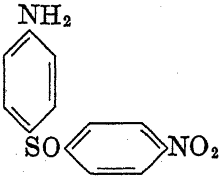
The reversing action of PABA on the antibacterial action of sulfones and sulfoxides was investigated by Levaditi and Pérault (231). With *E. coli* and *Staph. aureus*, they found that PABA prevented the action of bis(4-aminophenyl) sulfone and a number of related sulfones and sulfoxides. The action of antiseptics such as mercuric chloride, hydroxyquinolin, and thioflavine was not affected by PABA.



Bis(4-aminophenyl) sulfone



4-Aminophenyl-4′-hydroxyphenyl sulfone



4-Aminophenyl-4′-nitrophenyl sulfoxide

Bliss and Long (41) called attention to the fact that, in addition to PABA, other simple substances such as methionine also reverse the bacteriostatic action of sulfonamides under certain conditions. Of the ten amino acids tested with *E. coli*, only methionine consistently produced an antisulfonamide effect. With more potent sulfonamides, such as sulfapyridine and sulfathiazole, methionine was effective only against relatively small amounts of the sulfonamides. Even with sulfanilamide the reversal occurred over a relatively small range of concentrations, although very small amounts of methionine ($M/200{,}000$) prevented the bacteriostatic action of concentrations of sulfanilamide not exceeding $M/1000$. Above this concentration of sulfanilamide no amount of methionine was effective, so that, unlike the PABA relationship, no constant ratio was observed. The authors suggested that methionine might be a precursor of a substance with antisulfanilamide activity.

Of the many simple substances tested by Harris and Kohn (159), only methionine in addition to PABA was found to possess antisulfonamide activity. The *l*(−)-isomer was shown to be at least ten times as active as *d*(+)-methionine. In general, their results were in accord with those of Bliss and Long (41). Subsequently, Harris and Kohn (160, 203) reported that under certain conditions guanine and xanthine enhanced the effect of methionine. In the absence of methionine, however, these purines increased the antibacterial activity of sulfanilamide.

Snell and Mitchell (385) found that less PABA was required to reverse the action of sulfanilamide on certain lactic acid bacteria, if one of the purines (adenine, guanine, hypoxanthine, or xanthine) was added to the medium. Methionine was without effect in these experiments when *L. arabinosus* was the test organism. The purines and methionine were capable of at least partially replacing PABA as a growth factor for lactobacilli.

Kohn and Harris (159, 202, 203) extended the concept of Woods and Fildes

(122, 443) to incorporate the secondary reversing agents, such as methionine and the peptones. From quantitative data on *E. coli* obtained in both simple and complex media, they demonstrated that the size of the inoculum had no real effect on the bacteriostatic action of the sulfonamides. At normal bacteriostatic levels the primary inhibition appeared to be directed against growth rather than respiration. In synthetic media there was a simple relationship between rate of growth and sulfonamide concentration. The relationship in a proteose–peptone medium was more complex. These observations suggested that the sulfonamides interfere with the synthesis of substances necessary for growth. Kohn and Harris assumed that among these substances was a special group X requiring PABA as a catalyst. X was considered to represent a class of substances some of which were present in the proteose–peptone medium. Thus, low concentrations of sulfonamides were ineffective in this medium but higher concentrations produced bacteriostasis by interfering with additional syntheses. Methionine was assumed to act as a reversing agent, because the sulfonamides prevented its synthesis. The synthesis of methionine in turn was assumed to be dependent on the presence of PABA.

The competitive nature of the relationship between sulfonamides and PABA was investigated quantitatively by Wyss (461) and Wood (441). By mathematical analysis of his data, Wyss demonstrated that sulfanilamide and PABA appeared to compete for the same receptor site in the organism, the great disproportion between the molar concentrations of the metabolite and the antagonist merely indicating the greater affinity for PABA.

Using a different mathematical treatment, Wood (441) arrived at the same conclusions for sulfapyridine, sulfathiazole, sulfadiazine, sulfaguanidine, and bis(4-aminophenyl) sulfone as well as sulfanilamide. He also suggested that the variation in the antibacterial activity of the different compounds might be due to the difference in their affinity for the enzyme associated with PABA.

Ivanovics (183) examined the relation of drug resistance to the production of antisulfonamide substances. As indicated in table 3, he found that some resistant strains of *Staph. aureus* produced more sulfonamide-reversing agent than the parent strain. In other cases no marked differences could be detected. Where the differences were small, however, the inhibition ratio of sulfonamide to PABA changed considerably. Thus, while one molecule of PABA reversed the antibacterial action of only 14 molecules of sulfamethylthiazole with parent strain B, 220 molecules of the sulfonamide were reversed with the resistant strain B. On the basis of these observations, Ivanovics suggested that sulfonamide resistance could result either from an increased production of PABA (or a related substance), or by an increased efficiency in the utilization of PABA. In some instances, e.g., strain VI, a combination of both mechanisms might be involved.

In studying the bacteriostatic effect of sulfanilamide and four of its derivatives (sulfaguanidine, sulfapyridine, sulfathiazole, and sulfadiazine) on *E. coli*, Rose and Fox (350) observed that a constant concentration of PABA was required to reverse the minimum effective concentration (M.E.C.) of the various sulfonamides. Thus, although the M.E.C. of the different derivatives varied over 500-fold (i.e., from $2.5 \times 10^{-3}M$ for sulfanilamide to $4 \times 10^{-6}M$ for sulfathiazole and sulfadiazine), the same minimum concentration of PABA ($5 \times 10^{-7}M$) was required to prevent the bacteriostatic action of each at its M.E.C. Rose and Fox also found that the relationship of PABA to sulfathiazole was independent of the number of bacteria. The organisms appeared to undergo a definite limited number (six to seven) of cell divisions, regardless of the size of the original inoculum. They attributed these results to the inability of the organism to synthesize a substance, possibly involving PABA, and necessary for normal reproduction. The presence of the sulfonamide was assumed to prevent this synthesis so that, after a certain number of cell divisions, the concentration per individual cell was insufficient to permit further reproduction.

TABLE 3

*Relationship of PABA to sulfamethylthiazole with various strains of Staph. aureus**

Staph. STRAIN	TITER†	INHIBITION RATIO‡	ANTISULFONAMIDE ACTIVITY OF EXTRACT§
B (parent)	*M*/323,000	14	−
B (resistant)	*M*/10,000	220	−
VI (parent)	*M*/550,000	7	−
VI (resistant)	*M*/15,000	133	±
IX (parent)	*M*/480,000	31	
IX (resistant)	*M*/21,400	66	
X (parent)	*M*/60,000	21	−
X (resistant)	*M*/8,300	35	++

* Adapted from Ivanovics (183).

† Minimum effective concentration of sulfamethylthiazole.

‡ Moles of sulfamethylthiazole (*M*/1000) reversed by one mole of PABA.

§ − = absence of detectable amounts of PABA or other antisulfonamide; ± and ++ = relative antisulfonamide activity of extracts from various strains.

TABLE 4

Relationship of PABA to sulfanilamide (SA) and sulfathiazole (ST) with various organisms (463)

ORGANISM	MOLES SA/PABA	MOLES ST/PABA	EFFICIENCY ST/SA
E. coli	2,000	27	74
A. aerogenes	3,220	45	72
Staph. aureus	4,660	53	88
Ps. aeruginosa	13,330	184	73
Sal. typhimurium	6,650	92	72
L. acidophilus	8,000	133	60
P. vulgaris	4,000	55	73

Wyss, Grubaugh, and Schmelkes (463) examined the specificity of various sulfonamides for different bacteria. These investigators found no indication that the relative effectiveness of sulfanilamide and sulfathiazole changed from one organism to another (table 4). Similar results were obtained when other sulfonamides were compared.

Fox and Rose (130) and Schmelkes, Wyss, Marks, Ludwig, and Strandskov (364) pointed out a relationship between the degree of dissociation of sulfonamides and their antibacterial activity. With four sulfonamides, Fox and Rose demonstrated that while the molar ratio of PABA to sulfonamide varied over 600-fold, the ionic ratios were 1.4 for sulfanilamide and 1.4, 4.9, and 6.4 for sulfapyridine, sulfathiazole, and sulfadiazine, respectively. At pH 7.8 sulfanilamide was found to be about ten times as active as at pH 6.8, corresponding to the increase in the ionic form at the higher pH level. These observations led to the suggestion that only the ionic form of the sulfonamides possessed antibacterial activity. Similar results were obtained by Schmelkes *et al.* They also pointed out the relative activity of sulfonamides at different pH levels and concluded that the anionic form was the effective agent, since it might be expected to compete with PABA ions on a more favorable basis.

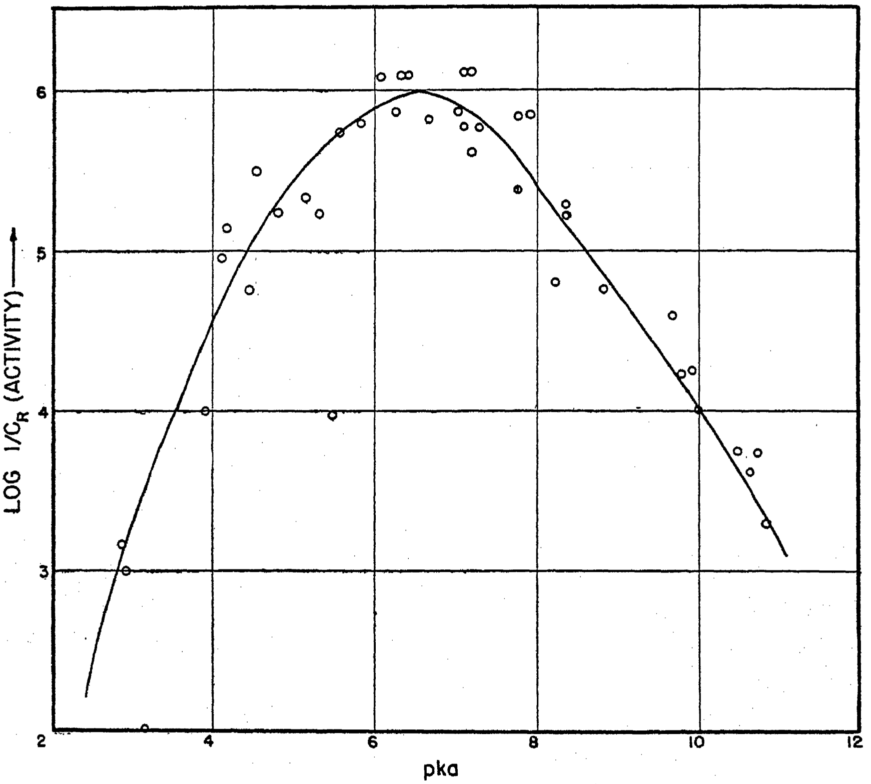
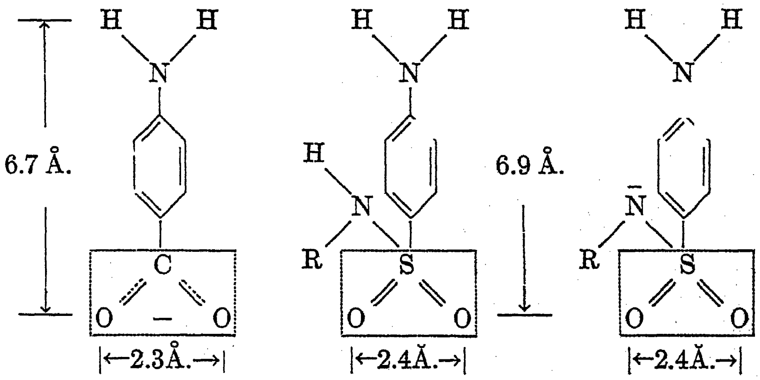


FIG. 2. The relationship between bacteriostatic action and acidity of N^1-substituted sulfanilamides. Bell and Roblin (32).

For several sulfonamides, Cowles (78) and Brueckner (51) found that antibacterial activity was at a maximum when the sulfonamide was approximately

50 per cent ionized. Cowles suggested that, like a number of other substances, the sulfonamides might penetrate the bacterial cell effectively only in the unionized form. Once inside the cell, the ionized form was considered to be responsible for the antibacterial action. Thus, the maximum activity of any sulfonamide would be expected to fall near the point at which pK_a = pH of the culture medium. At the point of 50 per cent ionization, all sulfonamides were regarded as essentially equally active. With the exception of sulfaguanidine[5], Cowles's experimental data appeared to be consistent with these views[6]. Brueckner confirmed Cowles's results and the earlier observations of Lwoff *et al.* (245), indicating that the efficiency of PABA in reversing sulfonamides decreases with increasing alkalinity of the culture medium in the range pH 6.0–8.9. He pointed out that this effect would explain the observation that the sulfonamide–PABA ratios decreased in this pH range for weakly acidic sulfonamides but remained relatively constant over most of the range for the more strongly acidic derivatives.

Bell and Roblin (32, 340) proposed a theory which related the structure of sulfanilamide-type compounds to their *in vitro* antibacterial activity. This theory was based on the experimental observation that when the acid dissociation constants of N^1-substituted sulfanilamide derivatives were plotted against activity a smooth curve was obtained which passed through a maximum as the acid strength increased (figure 2). The numerical values for some representative sulfonamides are recorded in table 5. It was suggested that if the bacteriostatic action of sulfonamides is due to a competition with PABA, then the more closely the competitor compound resembled the metabolite, the greater should be its blocking or bacteriostatic effect. The authors pointed out the similarity in the geometric configuration of sulfonamides and of *p*-aminobenzoate ion, as indicated:



p-Aminobenzoate ion Sulfonamide Sulfonamide ion

---

[5] The values for sulfaguanidine reported by Cowles (pK_a = 11.2) and Brueckner (pK_a = 10) do not appear to be correct (32). The acid strength is probably considerably weaker than these values indicate.

[6] While the experimental observations of Cowles (78) and Brueckner (51) are in accord with the idea that all sulfonamide ions are essentially equally active, the results of Fox and Rose (130) and Schmelkes *et al.* (364) indicate that as pK_a decreases the ratio PABA/sulfonamide ion also decreases (32, 340).

At pH 7, PABA exists almost exclusively in the dissociated form carrying a formal ionic change. Since the CO_2 ion is a strong negative group, the more negative the SO_2 group, the more closely it should resemble the CO_2 ion. The theory was stated as follows: *The more negative the SO_2 group of an N^1-substituted sulfanilamide derivative, the greater the bacteriostatic activity of the compound.* Bell and Roblin showed that the acid constants provided an indirect measure of the negative character of the SO_2 group. They demonstrated that, although the SO_2 group in the ionized form is more negative, the negative character of both forms decreases with decreasing pK_a. The net result of these two opposing factors resulted in a maximum in the experimental curve (figure 2). The theory was also developed mathematically and a theoretical curve obtained which agreed very well with the experimental results over a large part of the pH range. In general, from the known relative electronegative character of the N^1-substituents, it was possible to predict the *in vitro* activity of new sulfanilamide derivatives of this type. The theory also implied that the optimum in bacteriostatic action of sulfanilamide-type compounds appeared to have been reached.

TABLE 5

Dissociation constants and bacteriostatic activity of sulfanilamide-type compounds (32)

COMPOUND	pK_a*	K_a	$C_R(M \times 10^5)$†
N^1-Methylsulfanilamide	10.77	1.7×10^{-11}	30
Sulfanilamide	10.43	3.7×10^{-11}	20
N^1-*o*-Tolylsulfanilamide	9.96	1.1×10^{-10}	10
N^1-Phenylsulfanilamide	9.60	2.5×10^{-10}	3.0
5-Sulfanilamido-2-aminopyridine	8.82	1.5×10^{-9}	2.0
Sulfapyridine	8.43	3.7×10^{-9}	0.6
3-Sulfanilamidopyridine	7.89	1.3×10^{-8}	0.2
Sulfathiazole	7.12	7.6×10^{-8}	0.08
Sulfadiazine	6.48	3.3×10^{-7}	0.08
Sulfapyrazine	6.04	9.1×10^{-7}	0.08
Sulfamethylthiadiazole	5.45	3.5×10^{-6}	0.2
Sulfanilylurea	5.42	3.8×10^{-6}	10
Sulfacetimide	5.38	4.2×10^{-6}	0.7
Sulfathiadiazole	4.77	1.7×10^{-5}	0.6
3-Sulfanilamido-4-methylfurazan	4.10	7.9×10^{-5}	1.0
N^1-Chloroacetylsulfanilamide	3.79	1.6×10^{-4}	10
N^1-Ethylsulfonylsulfanilamide	3.10	7.9×10^{-4}	1000
Sulfanilylcyanamide	2.92	1.2×10^{-3}	100

* $pK_a = \text{Log } 1/K_a$.

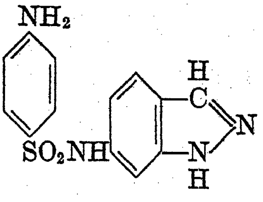
† C_R = minimum effective molar concentration for complete bacteriostasis of *E. coli* in synthetic medium buffered at pH 7.

Klotz (197) applied the law of mass action to a mathematical analysis of equilibria between PABA–enzyme complex and sulfonamide-enzyme complex with the dissociated forms of the metabolite and antagonists. Utilizing the data of Bell and Roblin (32), he arrived at similar conclusions regarding the optimum activity and the effect of changes in the pH of the culture medium. From quantitative studies with *E. coli*, Klotz and Gutmann (198) calculated the dissociation constants of the theoretical enzyme–sulfonamide complex for several derivatives. They considered that the deviations between predicted and observed bacteriostatic activities for sulfonamides of low pK_a might be due to extensive binding of the ionized forms of the compounds to extraneous bacterial proteins.

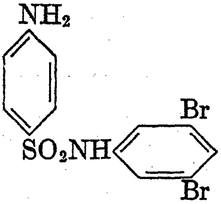
Although PABA usually produces a complete reversal of the growth-inhibiting action of practically all sulfonamides with a wide variety of microörganisms, several cases have been reported which constitute exceptions to this generalization. Thus, Feldt (119) and Hawking (167) demonstrated that the protective action of sulfapyridine and sulfathiazole on treponema infections in mice was not reversed by PABA. A number of other sulfonamides were ineffective as therapeutic agents. Hawking concluded that the effect of sulfapyridine and sulfathiazole on these infections was atypical.

Tamura (408) found that PABA failed to prevent the *in vitro* antibacterial action of sulfathiazole, sulfadiazine, or sulfapyrazine for *Bacterium tularense*. In fact, PABA itself inhibited the growth of this organism in concentrations exceeding *M*/10,000. Although lower concentrations did not produce a reversal, the usual action of PABA was observed with the same sulfonamides in the same medium when *E. coli* or *P. pestis* were the test organisms.

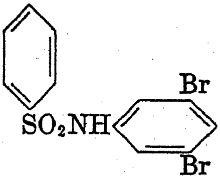
During the study of a group of isomeric sulfanilamidoindazoles (212), Lawrence and Goetchius (223) found that PABA only partially reversed the antibacterial action of these sulfonamides on *Brucella melitensis*. There was over a thousandfold decrease in the effect of PABA on these sulfonamides compared with sulfathiazole. Similarly, Goetchius and Lawrence (144) reported that the antibacterial activity of at least two members (sulfanilyl-3,5-dibromanilide and benzenesulfon-3,5-dibromoanilide) of a series of halogen-substituted sulfonanilides (193) was not appreciably affected by PABA[7].



6-Sulfanilamido-
indazole



Sulfanilyl-3,5-
dibromoanilide

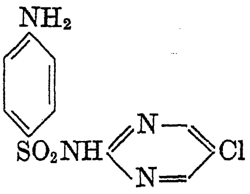


Benzenesulfon-3,5-
dibromoanilide

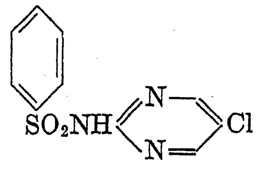
Brackett, Waletzky, and Baker (48) demonstrated that the antimalarial activity of 2-sulfanilamido-5-chloropyrimidine and of the corresponding bromo compound (109, 344) was only partially prevented by PABA, while the lesser activity of 2-benzenesulfonamido-5-chloropyrimidine was entirely unaffected by

---

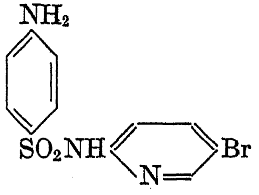
[7] White (432) found that these results require qualification, since the antibacterial action of sulfanilyl-3,5-dichloroanilide and the corresponding bromo derivative against certain Gram-negative organisms is completely reversed by PABA. The failure of PABA to prevent the growth inhibition caused by these compounds appears to be limited primarily to Gram-positive species.



2-Sulfanilamido-5-chloropyrimidine



2-Benzenesulfonamido-5-chloropyrimidine



2-Sulfanilamido-5-bromopyridine

this metabolite. On the other hand, PABA completely reversed the activity of other heterocyclic halogen compounds such as pyridine (343) and thiazole (109) derivatives. The authors concluded that two modes of action might be involved: one attributed to the anti-PABA effect of the sulfanilyl group, the other to the sulfonamidochloropyrimidine.

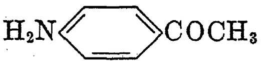
2. Other PABA antagonists

In addition to the sulfanilamides and other sulfur derivatives, a number of sulfur-free compounds chemically related to PABA have been found to inhibit the growth of microörganisms. The metabolite also reverses the antibacterial action of these compounds.

During a study of the action of sodium *p*-nitrobenzoate on a strain of *Strep. viridans*, Miller (292) observed that small concentrations (10–100 mg./100 cc.) of this substance were bacteriostatic, while larger amounts (200–400 mg./100 cc.) produced little or no effect. He suggested that part of the *p*-nitrobenzoate might be reduced to PABA by the organisms, and showed that the amount of diazotizable amine formed was proportional to the concentration of nitro compound. A filtrate from organisms grown in the presence of *p*-nitrobenzoate prevented the bacteriostatic action of sulfathiazole, whereas a similar filtrate lacking the nitro derivative did not (293). Miller (292) also demonstrated that the antibacterial activity of *p*-nitrobenzoate could be prevented by small amounts of PABA. This reversing action was confirmed by Ivanovics (184) with *Staph. aureus*, but he was unable to eliminate the bacteriostatic action of the nitro compound with PABA when *E. coli* was the test organism.

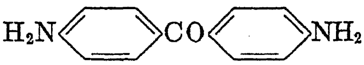
Kuhn, Möller, Wendt, and Beinert (206, 207) investigated the effects of a number of *p*-aminophenyl derivatives on *Streptobacterium plantarum*, an organism which requires preformed PABA for growth in a synthetic medium. Several derivatives could, to some extent, replace PABA, while *p*-aminobenzamide was either a growth factor or an inhibitor depending on the concentration. Hirsch (175) also found that the growth of *E. coli*, inhibited by *p*-aminobenzamide, could be restored by PABA. The corresponding thioamide was a growth inhibitor, but Kuhn *et al.* (207) concluded that the thioamide competed with the organisms for iron, since its bacteriostatic effect could be reversed by iron salts but not by PABA. Some of the results obtained by Kuhn and his associates are summarized in table 6.

It is evident that there are marked differences in the bacteriostatic activity of the various ketones. As the authors pointed out (207), the introduction of a



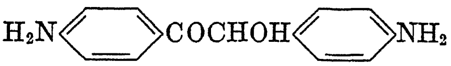
I

p-Aminoacetophenone



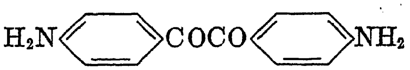
II

p,p′-Diaminobenzophenone



III

p,p′-Diaminobenzoin



IV

p,p′-Diaminobenzil

second carbonyl group (*cf.* II and IV) produces a thirtyfold increase in effectiveness. Over a short period of time III was also bacteriostatic. However, the activity dropped off rapidly with time, and Kuhn *et al.* (207) suggested that a "self-inhibition" caused by the formation of PABA might occur. This effect is similar to the results obtained by Miller (292), and analogous to the formation of benzoic acid by the autoöxidation of benzoin in alkaline solution.

TABLE 6

Relative activity of some p-aminophenyl derivatives as PABA antagonists (206)

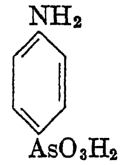
CONCENTRATION FOR 50 PER CENT INHIBITION	COMPOUND	REVERSAL
mg./100 cc.		*moles per mole* PABA
150	2-(*p*-Aminobenzamido) pyridine	48,000
30	Phosphanilic acid	12,000
25	*p*-Aminoacetophenone	12,700
10	Sulfanilic acid	5,000
7	*p*-Nitroacetophenone	2,900
5	*p,p′*-Diaminobenzophenone	1,600
0.3	Sulfanilamide	150
0.15	*p,p′*-Diaminobenzil	50*
0.1	*p,p′*-Diaminodiphenyl sulfone	28

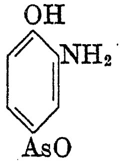
* Approximate value from reference 207.

Auhagen (21) corroborated the results of Kuhn and coworkers with I and found that *p*-aminobenzophenone was also a growth inhibitor. From the PABA reversal ratios the latter appeared to be the less active. He reported that II was slightly effective in the treatment of experimental streptococcal infections in mice.

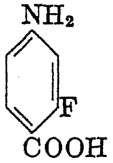
Green and Bielschowski (149) reported that the antibacterial action of 4,4′-diaminodiphenyl diselenide was partially reversed by PABA. *p*-Aminophenylseleninic and selenonic acids appeared to be too unstable to be prepared in sufficient purity for testing. Under certain conditions, PABA completely prevented the growth inhibition produced by 4,4′-diaminodiphenyl disulfide, but in some respects this substance seemed to differ from the sulfonamides. The authors concluded that the disulfide might act by two mechanisms, one of which was related to PABA metabolism.

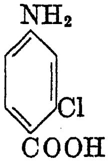
Arsenilic acid was studied by Hirsch (175), who reported that it reduced the speed of growth as measured by oxygen consumption. PABA counteracted this effect. Later, Peters (319) showed that $M/1000$ arsenilic acid was bacteriostatic for *E. coli*, and that this action could be prevented by $M/10{,}000{,}000$ PABA. Methionine also reversed the activity of arsenilic acid, as it does in the case of the sulfonamides. Peters found that PABA did not prevent the bacteriostatic action of arsenoxide, nor the trypanocidal action of either arsenilic acid or arsenoxide. He concluded that the trypanocidal action of arsenilic acid was exerted through the arsenoxide by a different mechanism from the bacteriostatic effect (*cf.* Section III, D).

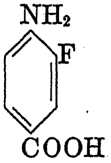

Arsenilic acid


Arsenoxide

A series of nuclear-substituted *p*-aminobenzoic acids prepared by Schmelkes and Rubin (363) was investigated by Wyss, Rubin, and Strandskov (464). 2-Fluoro-4-aminobenzoic acid (2-F-PABA) was found to be one-third as effective as PABA in growth factor tests with *Clostridium acetobutylicum*. This same substance reversed the bacteriostatic activity of sulfanilamide when *E. coli* was the test organism. In contrast to the 2-fluoro derivative, 2-Cl-PABA inhibited growth, and this inhibition was prevented by PABA or methionine. Similarly, the bacteriostatic action of 3-F-PABA was reversed by PABA. A number of other halogen-, amino-, and carboxy-substituted PABA derivatives were studied by Wyss and coworkers. 2-Cl-PABA appeared to be the most potent antibacterial agent, although it showed very little antipneumococcic activity in mice. The growth factor and antisulfonamide action of several other derivatives such as 2-Br-PABA was probably due to the presence of traces of PABA as a contaminant (39). However, it is unlikely that the high growth factor activity of 2-F-PABA could be explained on this basis.


2-F-PABA


2-Cl-PABA


3-F-PABA

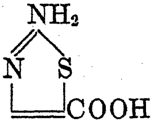
Results with a number of PABA derivatives were described by Johnson, Green, and Pauli (191). These investigators found that nuclear methyl and methoxy substituents resulted in compounds with bacteriostatic activity which PABA reversed. In general, they confirmed the observations of Wyss *et al.* (464) with other nuclear substituents, although they reported that 2-Cl-PABA exhibited antisulfonamide action with some microörganisms[8]. The effects of modifications of PABA in the benzene series were summarized as follows: (*1*) Monosubstitution by neutral or weakly electropositive groups yielded compounds with bacteriostatic properties. (*2*) Disubstitution in general resulted in compounds exhibiting no antibacterial action. (*3*) Replacement of the amino group, with the exception of the nitro group, produced inactive compounds. (*4*) Variation in the carboxyl gave antisulfonamide, bacteriostatic, or no activity. (*5*) Simultaneous modification of amino group and nuclear substitution, or changing both the amino and the carboxyl groups, yielded inactive compounds.

---

[8] The possibility that traces of PABA may have been formed in the synthesis of this compound does not appear to have been excluded.

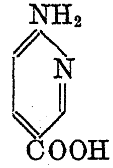
A similar group of nuclear-substituted derivatives, and of other compounds related to PABA, was investigated by Martin and Rose (278). Of the thirty-three compounds studied, 3-Cl-PABA and 3-HO-PABA had the highest anti-streptococcal activity. This activity was considerably reduced but not completely abolished by PABA. In addition, 2-methyl-PABA and 2-Cl-PABA were reported to have antisulfanilamide activity, while the 3-methyl derivative was inert. A slight chemotherapeutic effect in mice infected with streptococci or pneumococci was demonstrated for 3-HO-PABA.

Johnson and associates (191) also studied a group of heterocyclic analogs of PABA. Compound V was inactive, but VI was bacteriostatic for *E. coli* and streptococci in approximately the same concentrations as sulfapyridine. VII and its amide were active, whereas the corresponding furan derivative, VIII, showed no antibacterial action. The action of all the active heterocycles was reversed by appropriate concentrations of PABA. With *Strep. pyogenes*, Martin, Rose, and Swain (279) found IX to be without antibacterial activity, but it prevented the action of sulfanilamide on the same organism. PABA was approximately 2000 times as effective in this respect.



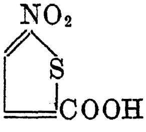
V

2-Amino-5-carboxythiazole



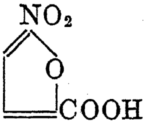
VI

2-Amino-5-carboxypyridine



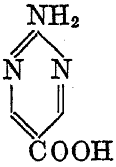
VII

2-Nitro-5-carboxythiophene



VIII

2-Nitro-5-carboxyfuran

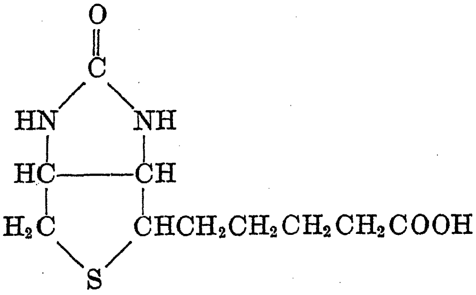


IX

2-Amino-5-carboxypyrimidine

C. Biotin

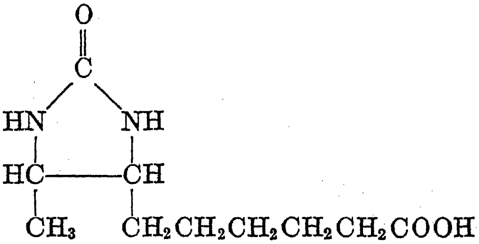
Although biotin as a constituent of "bios" was long recognized as a yeast growth factor, its identity with the anti-egg-white-injury factor (vitamin H) (152), its chemical constitution (419), and its synthesis (161, 162) are more recent developments. Alternative formulas have been proposed for biotin from various sources (200), but the total synthesis has established the structure of liver biotin as follows:



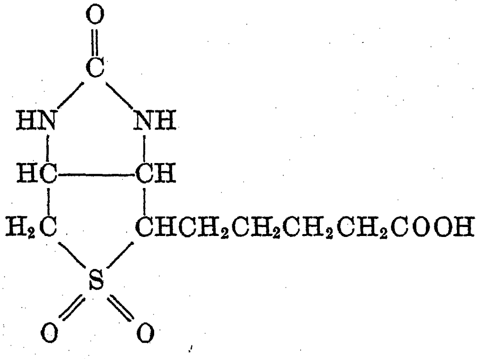
Biotin

The substance in egg white responsible for biotin deficiency has been shown to be a protein (98), to which the graphic name avidin has been assigned. This material forms a stable complex from which biotin cannot be readily dissociated. By the use of avidin it has been demonstrated that many bacteria probably require preformed biotin for growth (219).

Desthiobiotin was first obtained from biotin by cleavage with Raney nickel (421), although the total synthesis of the racemic derivative has also been described (440). *d*-Desthiobiotin was reported (286) to replace biotin for a strain of yeast but not for *Lactobacillus casei*. Subsequently, Dittmer, Melville, and du Vigneaud (87) found that the yeast strain appeared to synthesize biotin from the desthio derivative. These investigators and Lilly and Leonian (233) also found that desthiobiotin inhibited the utilization of biotin by *L. casei*. Dittmer and coworkers showed that growth due to 0.82×10^{-10} molar biotin was reduced



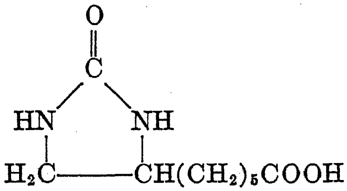
Desthiobiotin



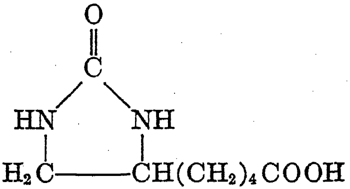
Biotin sulfone

to one-half of this value by 2.3×10^{-6} molar *d*-desthiobiotin. This growth inhibition was completely reversed by increasing the concentration of biotin to 4.1×10^{-10} molar. The results obtained by Lilly and Leonian were similar over a wide range of concentrations when *L. casei* was employed as the test organism. A number of other biotin-requiring organisms, most of which were found to utilize desthiobiotin as a growth factor, were also studied (233). In only one or two other cases did desthiobiotin exhibit antibiotin activity.

Dittmer and du Vigneaud (85) investigated the sulfone of biotin, which had been shown to possess slight biotin activity for a strain of yeast (86). With *L. casei*, *L. arabinosus*, and *Staph. aureus*, however, biotin sulfone inhibited growth. The antibiotin activity of this compound for *L. casei* was such that the molar inhibition ratio (moles of antagonist required to reduce growth stimulated by one mole of biotin to one-half of normal) was approximately 280. The growth inhibition was completely reversed by the addition of more biotin. Two analogues of desthiobiotin, 4-(imidazolidone-2)caproic acid and 4-(imidazolidone-2)valeric acid, were also prepared by Dittmer and du Vigneaud (85).



Imidazolidonecaproic acid

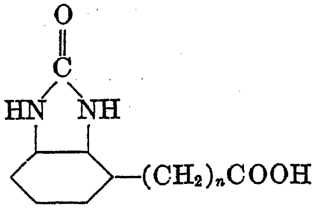


Imidazolidonevaleric acid

The caproic acid derivative was found to have antibiotin activity for both *L. casei* and a yeast, *S. cerevisiae*. The molar inhibition ratio, which was 9,100 for *d*-desthiobiotin with *L. casei*, was 126,000 with this organism for imidazolidonecaproic acid. In contrast to this result, imidazolidonevaleric acid stimulated the growth of yeast slightly, but it neither stimulated nor inhibited *L. casei*.

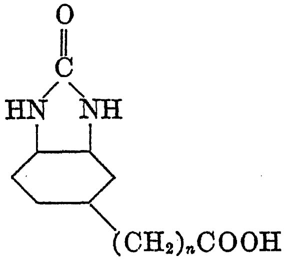
Rubin, Drekter, and Moyer (357) found that *dl*-desthiobiotin had about one-half the growth-inhibitory activity of the *d*-isomer for *L. casei*, suggesting that the *l*-desthio derivative was without antibiotin activity. The oxygen analogue of biotin, "oxybiotin" (177), also reversed the antibiotin activity of *dl*-desthiobiotin (358). In this instance, the inhibition ratio for half normal growth was 1200, as compared with 17,000 when biotin itself was the reversing agent.

A series of ureylenecyclohexane derivatives was synthesized by English, Clapp, Cole, Halverstadt, Lampen, and Roblin (108). In the type I series the cyclohexylvaleric acid derivative differed from biotin only in that the sulfur atom was replaced by two methylene groups, while in the type II compounds the side chain also was in a different position with respect to the urea ring. With few exceptions these compounds possessed antibiotin activity for both *L. casei* and yeast. With the former organism, γ-(3,4-ureylenecyclohexyl)butyric acid was the most active, 6×10^{-7} *M* producing a 50 per cent inhibition of growth. On the other hand, δ-(2,3-ureylenecyclohexyl)valeric acid was 100 times more effective as a growth inhibitor for yeast than the 3,4-butyric acid derivative. The molar inhibition



I

ω-(2,3-Ureylenecyclohexyl)-butyric or valeric acid



II

ω-(3,4-Ureylenecyclohexyl)-butyric or valeric acid

ratio in this case was 1500 for one-half normal growth. In all instances the growth-inhibitory action was completely reversed by appropriate concentrations of biotin. Geometrical isomers of the type I analogues were separated, but there appeared to be no significant difference in antibiotin activity between them. The analogous ureylenebenzene derivatives were also studied by English *et al.* The antibiotin activity of these compounds was considerably lower than that of the corresponding derivatives in the cyclohexane series. None of the products produced any growth stimulation with either of the organisms studied. The relative growth-inhibitory activity of a number of the compounds is summarized in table 7.

TABLE 7

Relative antibiotin activity of ureylenebenzene and ureylenecyclohexane derivatives†*

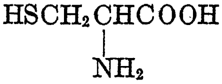
TYPE	*n*	CARBOCYCLIC RING	*L. casei*	YEAST
I	3	Phenyl	1	20
I	3	Cyclohexyl	4	4000
I	4	Phenyl	4	2.5
I	4	Cyclohexyl	800	2000
II	3	Phenyl	16	1
II	3	Cyclohexyl	6000	40
II	4	Phenyl	32	4
II	4	Cyclohexyl	800	40

* Arbitrary values based on least active derivative = 1 for each organism.
† Adapted from English *et al.* (108).

D. Glutathione and other mercapto compounds

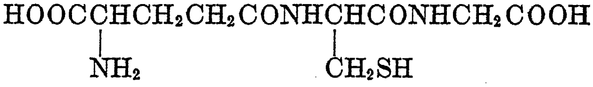
Enzyme proteins very often contain mercapto groups which are essential for enzymatic activity. Compounds of arsenic or selenium, the heavy metals, oxidizing agents, reactive alkyl halides such as iodoacetic acid (in short, most substances which react readily with —SH groups) have been employed to determine their presence in and necessity for the action of isolated enzymes. Several excellent reviews are available on this subject (34, 35, 168, 169). It is evident from these, and from more recent studies (28, 29, 379), that numerous enzyme systems involved in carbohydrate, nitrogen, and fat metabolism contain essential mercapto groups.

Simpler metabolites such as cysteine and glutathione (γ-glutamylcysteinylglycine) also owe many of their biochemical properties to the presence of a mercapto



$HSCH_2CH(NH_2)COOH$

Cysteine



$HOOCCH(NH_2)CH_2CH_2CONHCH(CH_2SH)CONHCH_2COOH$

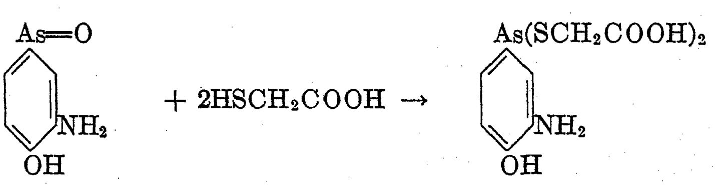
Glutathione

group. In fact, it has been suggested that the primary function of glutathione in cellular systems is the continuous reactivation of —SH enzymes. Glutathione is itself a coenzyme for the coglyoxalase enzyme system (406), although other similar substances, such as isoglutathione (α-glutamylcysteinylglycine) or asparthione (β-aspartylcysteinylglycine), can also serve in its place (31). Many microörganisms require an outside source of potential organic mercapto groups, and glutathione has been shown to be a growth factor for variant strains of the gonococcus (146).

Ehrlich (110) first suggested in 1909 the possibility that the toxic action of arsenicals might be due to their affinity for mercapto groups. About the same time, Chick (65) demonstrated that the action of mercuric chloride on bacteria was reversed by excess hydrogen sulfide. It was not until 1923, however, that the importance of —SH compounds in relation to the trypanocidal action of arsenicals was established experimentally by Voegtlin, Dyer, and Leonard (423). The basis for their work was the concept, now generally accepted, that arsenicals are directly toxic only in the arsenoxide form. Compounds in other stages of oxidation were presumed to be converted to arsenoxides before exerting trypanocidal action, i.e.,

$$RAsO_3H_2 \rightarrow RAs{=}O \leftarrow RAs{=}AsR$$

Voegtlin *et al.* (423) applied the known reactivity of arsenoxides with hydrogen sulfide to 3-amino-4-hydroxyphenylarsenoxide (arsenoxide) and mercaptoacetic acid, which were shown to react as follows:



I II

Trypanosomes and other cells with an active metabolism were found to contain —SH groups, as indicated by the characteristic nitroprusside test. *In vitro* tests demonstrated that, whereas *M*/20,000 arsenoxide (I) killed trypanosomes in less than 15 min., the organisms retained their motility for a period comparable with the controls in *M*/200 arsenoxide together with *M*/20 mercaptoacetic acid.

Cysteine, glutathione, and other mercapto derivatives also prevented the toxicity of I, but a number of other amino acids not containing sulfur were without effect. The disulfides of the active —SH compounds were either much less effective or devoid of reversing action. Experiments carried out *in vivo* with infected rats gave similar results. In this case, a ratio of mercapto compound to I of about 100:1 was required to abolish the action of the latter. A considerable lag in therapeutic effect was observed in tests with the thioarsenite (II). The reversible nature of the reaction

$$RAs{=}O + 2HSR' \leftrightharpoons RAs(SR')_2 + H_2O$$

was suggested as an explanation for these observations. Voegtlin and coworkers also demonstrated that the mercapto compounds were readily oxidized to the less active disulfides under their experimental conditions. This factor would also increase the amount of antagonist required.

Rosenthal and Voegtlin (352) found that glutathione protected rats from an otherwise lethal dose of arsenoxide. They also demonstrated that trypanosomes exposed to arsenoxide plus glutathione for an hour retained their infectivity for animals as well as their motility, whereas with arsenoxide alone the organisms lost both infectivity and motility after 5 min. exposure. Similar results were obtained by Reiner and Leonard (335) with sodium mercaptoacetate.

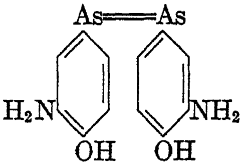
The trypanocidal action of several thioarsenites synthesized by Cohen, King, and Strangeways (72, 73) was investigated by Strangeways (403). The thioarsenites, prepared from aromatic arsenoxides and gluthathione or cysteine, were equally as toxic as the parent arsenoxides. Dilutions as high as 1:25,000,000 killed trypanosomes in 6 hr. This activity was attributed to the hydrolysis of the thioarsenites, which had been shown to be favored by alkali (72). Strangeways (403) pointed out that dilution would also favor the right-hand side of the equation,

$$RAs(SR')_2 + 2H_2O \leftrightharpoons RAs(OH)_2 + 2R'SH$$

whereas excess mercapto compound should shift the equilibrium to the left. Thus, an excess of glutathione had no preventive effect on the trypanocidal action of high dilutions (1:1,000,000) of either the arsenoxides or thioarsenites, even when 2000 moles of glutathione was employed per mole of arsenical. When the concentration of the arsenicals was increased to $M/1000$ or greater, however, complete reversal was observed at molar ratios of only 10:1. The trypanocidal action of tartar emetic and acriflavine was not affected by glutathione or cysteine.

The reaction of glutathione and cysteine with copper sulfate and auric chloride was examined by Voegtlin, Johnson, and Dyer (424). In addition to the formation of copper and gold derivatives, part of the —SH compounds appeared to be oxidized, with the formation of cuprous and aurous salts. It was suggested that the toxic action of the metallic salts might be due to a disturbance of the equilibrium, $2RSH \leftrightharpoons RSSR + H_2$. Rats could be protected from a lethal dose of sodium cupritartrate with sufficient amounts of glutathione or cysteine. The toxic action of copper sulfate on spirogyra was also prevented by excess glutathione.

Eagle (97) extended the earlier observations in a study of the effect of mercapto compounds on the antispirochetal activity of arsenic, bismuth, and mercury compounds *in vitro*. He found that the immobilization of *T. pallidum* by substances such as arsenoxide, arsphenamine, and mercuric chloride was prevented by an excess of glutathione, cysteine, and sodium mercaptoacetate. Methionine and thiamine had no effect. In the case of glutathione and arsenoxide, 5–12 moles was required to inactivate one mole of arsenoxide, but Eagle considered that the hydrolysis of the thioarsenite (72, 403) probably explained the discrepancy.



Arsphenamine

The action of mercuric chloride on bacteria (*E. coli*) was examined by Fildes (123) from the same general point of view. He found that mercaptoacetate, cysteine, and glutathione effectively prevented the bacteriostatic action of mercuric chloride, whereas oxidized cysteine (cystine) and methionine as well as a number of other metabolites such as thiamine, nicotinamide, riboflavin, and pantothenic acid were ineffective. A molar ratio of approximately 4:1 for glutathione and 25:1 for mercaptoacetate was required to produce complete reversal. These differences were explained on the basis of the relative chemical stability of the products formed when these two mercapto compounds combined with mercuric chloride, i.e.,

$$2RSH + HgCl_2 \leftrightharpoons (RS)_2Hg + 2HCl$$

Even when the organisms were exposed to mercuric chloride (2×10^{-7} *M*) for 4 days at room temperature, the addition of excess —SH compound allowed growth to proceed normally. On the other hand, higher concentrations (2×10^{-6} *M*) and temperatures (38°C.) for 17 hr. appeared to produce an irreversible effect on the bacteria. Fildes concluded that within limits the only function of mercuric chloride was to inactivate mercapto groups without other demonstrable injury to the cell. Several organic mercurials have also been shown to be inactivated by sodium mercaptoacetate (Nungester, Hood, and Warren (312)). Under the same conditions, other disinfectants such as phenol and iodine were not affected.

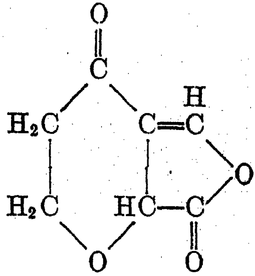
Albert, Falk, and Rubbo (8) studied the effect of arsenoxide and other arsenicals on several bacteria. They found that arsenoxide possessed considerable antibacterial action comparable to mercuric chloride, and that its bacteriostatic effect could also be reversed with an —SH compound, in this case mercaptoacetate. On the other hand, the pentavalent arsenical, *m*-acetylamino-*p*-hydroxyphenylarsonic acid, was ineffective, and neoarsphenamine showed only slight activity. Thus, at least one arsenoxide could be shown to possess considerable antibacterial action of the same type as that of the mercurials, while closely related arsenicals at higher or lower levels of oxidation were inactive.

Chen, Geiling, and MacHatton (64) found that cysteine reduced the trypanocidal activity and toxicity of trivalent antimonials such as tartar emetic, fuadin, anthiomaline, and antimony sodium thioglycolate, but not of the pentavalent derivatives, stibamine, stibenyl, and neostibosan.

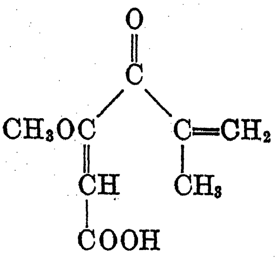
Substances preventing the action of an antibiotic agent called penicidin (derived from a penicillium but apparently not penicillin or clavicin) were investigated by Atkinson and Stanley (20). In addition to a slow-acting, dialyzable substance in serum, several mercapto compounds rapidly inactivated the antibacterial action of penicidin. Glutathione was the most effective, *M*/1000 reducing the activity approximately thirtyfold. Cysteine and mercaptoacetate were less effective, while other types of reducing agents showed no antagonistic action. When a mercapto compound and penicidin were mixed in solution and the nitroprusside test applied, the —SH group was found to disappear very quickly. This result favored the conclusion that a chemical reaction took place between the two substances, and that the antibiotic activity was due to the inactivation of essential mercapto compounds.

A number of other antibiotic agents were tested for bacteriostatic action in the presence of cysteine by Cavallito and Bailey (59). In every case examined (penicillin, citrinin, gliotoxin, clavicin, pyocyanine, and the active principles of several plants) cysteine diminished or completely prevented the antibacterial activity. The methyl and ethyl esters of cysteine were also effective in this respect, but *S*-methylcysteine, methionine, alanine, and serine were not. In some instances the inactivation was considered to be due to an irreversible reaction of cysteine with the antibiotic. Other —SH compounds, such as glutathione and mercaptoacetic acid, were much weaker or entirely ineffective. The action on Gram-positive organisms appeared to be more susceptible to cysteine inactivation than the Gram-negative activity. It was suggested that possibly the fundamental mode of action of certain classes of antibiotics involves their ability to interfere with the normal function of mercapto groups in bacterial metabolism.

A more detailed study of the preventive effect of mercapto compounds on the antibacterial activity of clavicin and penicillic acid was carried out by Geiger and Conn (139). Attention was called to the fact that these antibiotics are α,β-

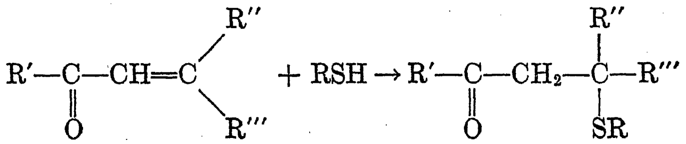


Clavicin



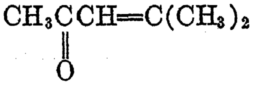
Penicillic acid

unsaturated ketones, which in general react with —SH compounds as follows:



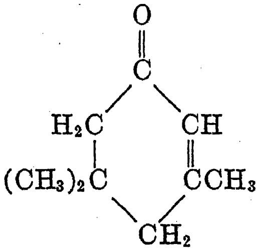
This reaction takes place most readily when R′ is an aromatic radical and R″ or R‴ is H.

Both clavicin and penicillic acid were inactivated when incubated for 24 hr. with an excess of mercaptoacetate or cysteine. Only a slight excess of mercaptoacetate (10 per cent) was required to inactivate clavicin almost completely. Thiosulfate also prevented the action of clavicin but not of penicillic acid. Tests were carried out with a number of different microörganisms, and the activity against the Gram-negative *E. coli* seemed to be more easily abolished than the effect on several Gram-positive organisms. Other α,β-unsaturated ketones, such as mesityl oxide, isophorone, indalone, benzalacetophenone, and acrylophenone, were tested for bacteriostatic activity.

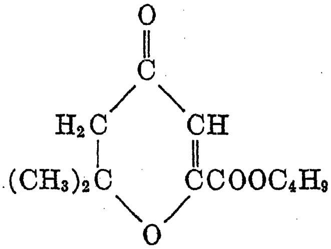


$\mathrm{CH_3}\underset{\underset{\mathrm{O}}{\|}}{\mathrm{C}}\mathrm{CH{=}C(CH_3)_2}$

Mesityl oxide



Isophorone



Indalone

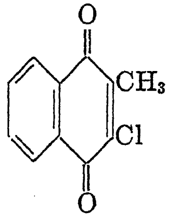
$C_6H_5COCH{=}CHC_6H_5$ Benzalacetophenone

$C_6H_5COCH{=}CH_2$ Acrylophenone

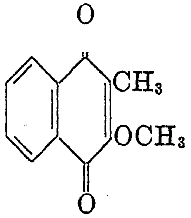
Of these, only acrylophenone showed a high degree of activity. The action of this compound was also prevented by mercaptoacetate and cysteine, whereas the lower activity of compounds such as benzalacetophenone was not. This difference was explained on the basis of the chemical reactivity of the unsaturated ketones. Clavicin, penicillic acid, and acrylophenone reacted rapidly and completely with mercaptoacetic acid, but benzalacetophenone reacted much more slowly and incompletely.

Chow and McKee (66) found that the inactivation of penicillin by cysteine was a function of the time of exposure. Thus, 0.02 mg. of penicillin was inactivated in 48 hr. by 0.08 mg. of cysteine, while 1.2 mg. was required to destroy the same amount of antibiotic in 5 hr. On the basis of these observations, it was concluded that the inactivation of penicillin was due to a chemical reaction rather than interference with an essential —SH enzyme system. Other amino acids—such as glycine, methionine, serine, and cystine—were inactive. Mercaptoacetic acid showed some effect, but simple mercaptans in general were not effective.

Colwell and McCall (74) investigated the effect of mercapto compounds on the bacteriostatic activity of naphthoquinones. They showed that sodium mercaptoacetate and cysteine hydrochloride prevented the antibacterial action of 2-methyl-1,4-naphthoquinone and its 3-chloro derivative, with *E. coli* as the test organism. On the other hand, the —SH compounds failed to affect the bacteriostatic action of 2-methyl-3-methoxy-1,4-naphthoquinone, indicating that an interference with essential mercapto groups might not completely explain the growth-inhibitory mechanism of this class of compounds.



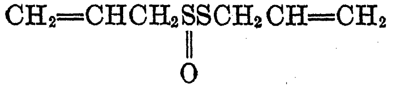
2-Methyl-3-chloro-
1,4-naphthoquinone



2-Methyl-3-methoxy-
1,4-naphthoquinone

Streptomycin, but not streptothricin, was found to be inactivated by cysteine (Denkewalter, Cook, and Tishler (82)). 2-Aminoethanethiol was also effective in preventing the antibiotic activity of streptomycin, but mercaptoacetic acid produced no significant effect. Cysteine-inactivated streptomycin could be quantitatively reactivated by iodine. The authors concluded that a mechanism postulating either a reversible chemical reaction between the antibiotic and certain —SH compounds, or a competitive effect on metabolic processes would be consistent with their observations.

A group of antibiotics, and other antibacterial agents which were inactivated by —SH compounds, were investigated by Cavallito, Bailey, Haskell, McCormick, and Warner (60). They concluded that the inactivation might be due to one of several mechanisms, and distinguished between specific and non-specific effects. Thus, penicillin was inactivated by only a few of the mercapto compounds tested, whereas allicin appeared to be inactivated by almost any —SH derivative. The mechanisms of reaction between antibacterial agents and mercapto compounds were enumerated as follows: (*1*) Oxidation—e.g., inorganic oxidizing agents, allicin, quinones, and certain dyes. (*2*) Formation of heavy metal complexes—such as the action of mercury, silver, and other metals forming mercaptides or complex addition products. (*3*) Metathesis—e.g., the reaction with alkylating agents such as iodoacetic acid. (*4*) Condensation—as in the formation of thioacetals or ketals with aldehydes and ketones[9]. (*5*) Addition to



$$CH_2{=}CHCH_2S\underset{\overset{\|}{O}}{S}CH_2CH{=}CH_2$$

Allicin

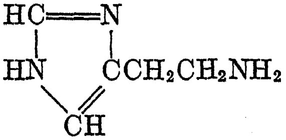
---

[9] Although the authors did not mention it, the formation of thioarsenites with arsenoxides

unsaturated structures,—such as clavicin and penicillic acid, and other unsaturated ketones or esters.

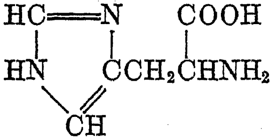
E. Histamine

Histamine is present in appreciable quantities in most body fluids and tissues. It is a substance of multifarious actions, minute amounts exerting a powerful vasodilator effect while contracting other smooth muscle. To account for the absence of these effects under normal conditions, histamine is generally assumed to occur in a bound form *in vivo*. Although its rôle has not been completely elucidated, the liberation of histamine appears to be an important factor in anaphylactic shock and many allergic conditions such as asthma and hay fever (36, 118, 151). Most vasoconstrictor substances counteract the vasodilator effect of histamine, and spasmolytic agents such as atropine neutralize the contracting action of histamine on smooth muscle (173). However, other agents have been found which, at least in some cases, appear to be more direct and specific histamine antagonists.

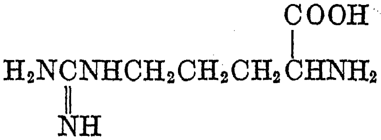


Histamine

Edlbacher, Jucker, and Baur (100) first demonstrated that arginine, histidine, and cysteine inhibit the effect of histamine on isolated intestinal strips of guinea pigs. They reported that Rothlin found the contracting action of acetylcholine on intestinal strips was not inhibited by arginine, indicating the specificity of the histamine antagonism. The other amino acids tested did not counteract the effect of histamine. The molar inhibition ratio for arginine appeared to be approximately 28,000:1, when the histamine action was completely prevented. Histidine and cysteine were reported to be somewhat less effective. In all cases the reaction was reversible, since, after removal of the antagonist, exposure to histamine again produced the usual contraction of the intestinal strips.



Histidine

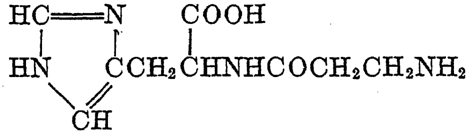


$$\mathrm{H_2N\underset{\underset{NH}{\|}}{C}NHCH_2CH_2CH_2\overset{\overset{COOH}{|}}{C}HNH_2}$$

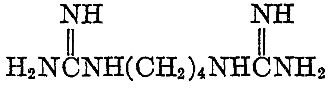
Arginine

The observations with histidine were confirmed by Mackay (268), who found that this substance inhibited the action of histamine *in vitro*. Histidine did not appear to influence the depressor action of histamine in the cat[10]. Mackay reported that the effect of acetylcholine on isolated gut strips was also prevented by histidine.

In an effort to correlate structure and activity, Ackermann and Wasmuth (3) carried out an extensive study of compounds which antagonized the action of histamine on isolated intestinal strips of guinea pigs. They reported that, in addition to arginine and histidine, combined forms of these substances such as arginine-rich proteins and carnosine were active inhibitors. Other guanidine derivatives and polyamines, such as arcaine, agmatine, γ-guanidobutyric acid, canavanine, spermidine, and spermine, also prevented the action of histamine.

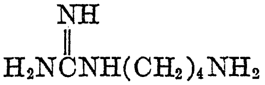


Carnosine



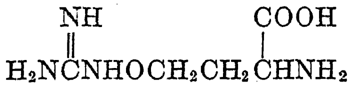
$$H_2N\overset{NH}{\overset{\|}{C}}NH(CH_2)_4NH\overset{NH}{\overset{\|}{C}}NH_2$$

Arcaine



$$H_2N\overset{NH}{\overset{\|}{C}}NH(CH_2)_4NH_2$$

Agmatine



$$H_2N\overset{NH}{\overset{\|}{C}}NHOCH_2CH_2\overset{COOH}{\overset{|}{C}}HNH_2$$

Canavanine

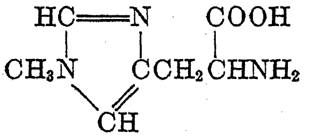
$H_2N(CH_2)_3NH(CH_2)_4NH_2$

Spermidine

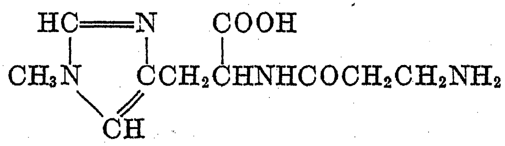
$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

Spermine

Since creatine, creatinine, and *asym*-dimethylguanidine were inactive, it was suggested that the presence of a free hydrogen atom on the side-chain nitrogen was necessary for activity. An acid group near the substituted hydrogen of a guanidine derivative, as in glycocyamine and β-guanidopropionic acid, appeared to eliminate activity. Similarly, methylation of the imino group, as in 1(3)-methylhistidine and anserine, destroyed the antagonistic action of the parent compounds.



1(3)-Methylhistidine



Anserine

On the basis of these studies, Ackerman and Wasmuth (1, 3) suggested that the NH group in the guanidines and secondary amines competed on a mass action basis with the ring NH of histamine for the receptor groups in smooth muscle. The ring NH was assumed to anchor the molecule in the tissues, while the toxic action of histamine was attributed to the primary amino group. Using gut strips from sensitized guinea pigs, they demonstrated that arginine prevented the contraction which would normally have been produced by the liberated histamine, without interfering with the desensitization of the strips. Ackerman and Wasmuth (2) reported that neither choline nor acetylcholine was inhibited by arginine.

Histidine hydrochloride was also studied by Halpern (155), who found that

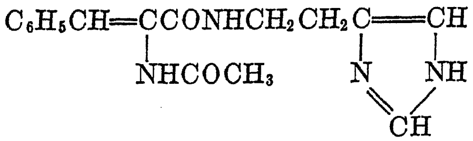
10 mg. of histidine completely and immediately counteracted the contraction of guinea pig gut produced by 1 γ of histamine—a molar inhibition ratio of approximately 6500:1. He reported that histidine caused a considerable immediate reduction in the tone and diminished the amplitude and frequency of the contraction of the isolated intestine of the rabbit. Histidine also prevented, although in a more irregular fashion, the action of acetylcholine, eserine, and barium chloride.

Landau and Gay (216) conducted a careful investigation of the influence of the monohydrochlorides of arginine and histidine on histamine and anaphylactic reactions of isolated smooth muscle and in intact guinea pigs. They also summarized the results in an earlier paper by Linneweh (236) on this subject. Linneweh extended the previous studies to other isolated smooth muscles, and found that arginine inhibited the histamine effect in every case. The effects of acetylcholine and epinephrine were not influenced by arginine. The action of pitocin was inhibited, although to a lesser extent than that of histamine. Guinea pigs were protected from histamine toxicity and anaphylactic shock with large doses of arginine monohydrochloride.

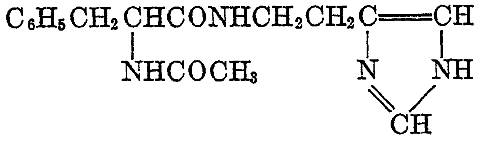
The investigations of Landau and Gay (216) also confirmed, in general, the earlier observations with both arginine and histidine. They found that the action of acetylcholine was not suppressed by concentrations of arginine or histidine which prevented histamine contractions of intestinal strips. In some cases, however, the response to acetylcholine was decidedly weaker in the presence of the antagonists, and it was concluded that absolute specificity for arginine or histidine as histamine antagonists for smooth muscle was not established. In some instances Landau and Gay also observed a relaxation of the tone of isolated muscle in the presence of arginine or histidine. They confirmed Linneweh's results with arginine by protecting guinea pigs from fatal doses of histamine, but were unable to extend the protection to anaphylactic shock. Higher doses, which might have produced positive results, were found to be too toxic.

The results of Gredner and Schumrick (147) and of Jadassohn, Fierz-David, and Vollenweider (187, 188) did not corroborate some of the observations in the preceding studies. The latter investigators reported that the antihistamine activity of arginine and the polyamines did not parallel their effect on the anaphylactic contraction of the guinea pig uterus. The antihistamine activity decreased in the order: arcaine, spermine, spermidine, and arginine. Triethylenetetramine showed only slight antihistamine activity, but it was a more potent inhibitor of anaphylactic contractions than arcaine. Both groups of investigators reported that the effect of these substances was not limited to histamine, since they also prevented the action of pitocin, choline, and acetylcholine. Gredner and Schumrick considered that the antihistamine activity was due to a slight antispasmodic effect, which counteracted any contractions regardless of the nature of the causative agent.

Rocha e Silva (345) prepared several simple peptide-like derivatives of histamine which he considered might serve as models for bound histamine, e.g.:



Acetyldehydrophenylalanylhistamine

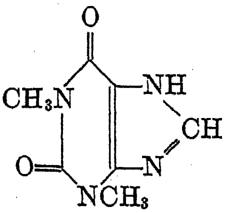


Acetyl-*dl*-phenylalanylhistamine

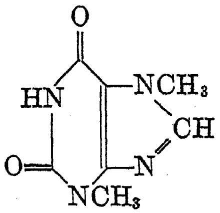
With the amino group of histamine blocked, the compounds were found to possess little or no pharmacological activity before hydrolysis. These observations led Rocha e Silva (346) to investigate the antagonistic action of these and some acyl derivatives of arginine. Several of the latter compounds were found to be more active than arginine as histamine antagonists, although in sufficient concentrations they also prevented the action of acetylcholine. Some of the results are summarized in table 8, together with molar inhibition ratios calculated from these data.

Morris and Dragsted (300) demonstrated that imidazole itself blocked the action of histamine on guinea pig intestinal strips in a ratio of about 1500:1 (partial inhibition). Doses of 1–128 mg./kg. in cats diminished, but did not completely prevent, the effect of histamine. The smaller amount reduced the effect approximately 25 per cent, whereas 128 mg./kg., which was the maximum tolerated, produced a 60 per cent reduction.

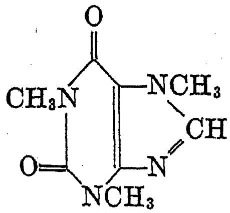
The antihistamine effects of xanthine and the methylated xanthines—theobromine, caffeine, and theophylline—were examined by Emmelin, Kahlson, and Linström (106). These investigators reported that theophylline monoethanolamine (1/1500) antagonized the action of histamine (0.15γ/10cc.) on smooth muscles. This substance and the other xanthines prevented the anaphylactic reaction in the bronchi of sensitized guinea pigs. The effects of acetylcholine were also antagonized, although the xanthine derivatives alone did not regularly or significantly influence bronchial tone. The same smooth muscle was not insensitive to all kinds of stimuli, since the contracting action of barium chloride was not antagonized. The authors concluded that the xanthines in some manner render smooth muscles insensitive to histamine.



Theophylline



Theobromine



Caffeine

Young and Gilbert (468) and Gilbert and Goldman (140) demonstrated that although theophylline–ethylenediamine (aminophyllin) alone had little effect on thin sections of rabbit or dog lung tissue, it had a definite protective effect against the constrictor action of histamine. The former authors found that a solution of 1:1000 aminophyllin in many of the trials completely prevented the bronchiole constriction produced by 1:50,000 histamine. Neither the sodium salt of theophylline (1:2000) nor the free acid (1:5000) showed any antihistamine activity. Ethylenediamine and alkalinity corresponding to that of the aminophyllin solution (pH 8.5) were also without effect. *In vivo* some degree of protection was obtained against fatal doses of histamine in guinea pigs, as well as a slight effect on anaphylactic shock in these animals.

TABLE 8

Relative amounts of different compounds required to inhibit the action of histamine on the guinea pig ileum (346)

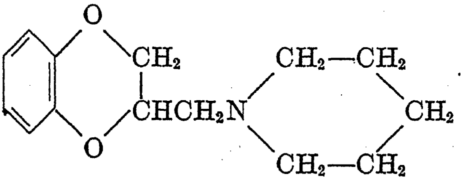
COMPOUND	AMOUNT OF M/20 SOLUTION	HISTAMINE ADDED	MOLAR INHIBITION RATIO†
	cc.	γ	
Arginine hydrochloride*	0.94	0.036	145,000
Benzoyl-*l*-arginine	0.35	0.02	97,000
Benzoyl-*l*-arginineamide hydrochloride*	0.18	0.033	30,000
Histidine hydrochloride*	0.18	0.03	33,000
Acetyldehydrophenylalanylhistamine hydrochloride*	0.29	0.043	37,500
Acetyl-*dl*-phenylalanylhistamine hydrochloride*	0.25	0.045	31,000

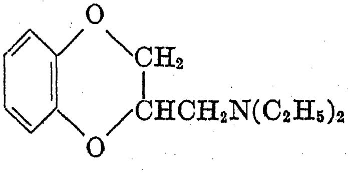
* Average of two or more values on different intestinal strips.

† Moles of antagonist required to inhibit one mole of histamine; calculated from Rocha e Silva's data (346).

Another group of substances, mainly derivatives of ethanolamine and ethylenediamine, has received considerable attention as histamine antagonists. While these compounds may not be specific in their action, some of them are powerful antagonists of many of the complex effects of histamine.

In 1937 Unger, Parrot, and Bovet (414) reported that a spasmolytic agent, piperidinomethylbenzodioxan, in a concentration of one part in 100,000, inhibited the action of histamine (1 γ/10 cc.) on the isolated intestine of the guinea pig. The action appeared to be specific and reversible (after washing, the gut


2-*N*-Piperidinomethyl-1,4-benzodioxan

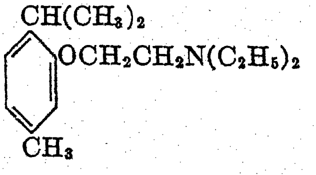

2-Diethylaminomethyl-1,4-benzodioxan

strip regained its normal reaction toward histamine). Later Vallery-Radot, Bovet, Mauric, and Holtzer (417, 418) studied the effect of this compound and the related diethylaminomethylbenzodioxan on anaphylactic and histamine shock in the rabbit. Under certain conditions, these substances protected the animals, the diethyl derivative acting more regularly than the corresponding piperidine.

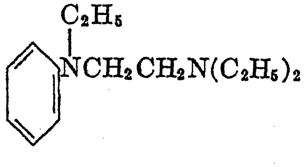
Several compounds in the phenoxyethyldiethylamine $[C_6H_5OCH_2CH_2N(C_2H_5)_2]$ series were investigated by Bovet and Staub (47), who found that 2-isopropyl-5-methylphenoxyethyldiethylamine (thymoxyethyldiethylamine), in particular, regularly protected guinea pigs from toxic doses of histamine. The parent compound, its *p*-methoxy derivative, β-naphthoxyethyldiethylamine, and diethylaminomethylbenzodioxan were less active, while α-naphthoxyethyldiethylamine and piperidinomethylbenzodioxan were reported to be almost inactive. Thymoxyethyldiethylamine was also the most active in antagonizing the effect of histamine *in vitro*. No close relationship between histamine antagonism and spasmolytic activity was found in this series of compounds. Thus, thymoxyethyldiethylamine had only a slight spasmolytic action and β-naphthoxyethyldiethylamine, none. The phenolic ethers possessed pharmacological properties more closely related to those of histamine itself. Bovet and Staub suggested that their action as histamine antagonists might be due to their respiratory stimulation. In another series, diethylaminoethylaniline, which was without marked pharmacological properties, showed antihistamine activity. The effect of thymoxyethyldiethylamine on anaphylactic shock in guinea pigs was studied by Staub and Bovet (398), who demonstrated a definite protective action with this compound both *in vitro* and *in vivo*.

Staub (396, 397) carried out a comprehensive investigation of a number of compounds in the phenoxyethyldiethylamine and diethylaminoethylaniline series. In comparing the relative activity of compounds, she preferred histamine shock to the isolated-gut method because of the variability of the latter. Thymoxyethyldiethylamine, the most active of the phenolic ethers, protected guinea pigs against 2 M.L.D.'s (minimum lethal doses) of histamine in doses of 5 mg./kg., although symptoms of shock were present. An equal dose of *N*-phenyl-*N*-ethyl-*N*′-diethylethylenediamine protected the animals against 3–4 M.L.D.'s and no shock symptoms were observed. In the same test atropine (5 mg./kg.) was effective against only 1 M.L.D. of histamine. Some of the relationships between structure and activity as recorded by Staub (396) are indicated in table 9.

A number of other structural modifications were also studied. Thus, in the thymoxyethyldiethylamine series (I) removal of one *N*-ethyl group eliminated



I

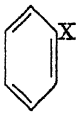
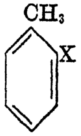
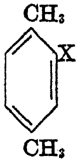
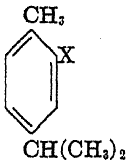


II

the activity, while replacing the diethylamino group by piperidine decreased the antihistamine index (A.H.I. = 2). Variations of *N*-phenyl-*N*-ethyl-*N'*-diethylethylenediamine (II), such as removal of the one *N*-ethyl or of both the *N'*-ethyl groups, destroyed activity. Replacing the *N*-ethyl by methyl reduced the A.H.I. to 1.5, and the *N*-isopropyl derivative was also less active than the *N*-ethyl derivative (A.H.I. = 3). The antihistamine activity of compounds I and II on smooth muscles of various organs was investigated by Staub (397). She found that 5–10 parts by weight of these derivatives prevented the action of one part of histamine on the isolated guinea pig gut. They also prevented the effect of histamine on the isolated guinea pig uterus and lung as well as the bronchi *in situ*, but were ineffective, in general, against the vascular and local skin reactions. A certain amount of parallelism between the relative activity against these different effects of histamine was found, but marked divergencies

TABLE 9

Antihistamine index of various diethyl derivatives of phenoxyethylamine and N-phenylethylenediamine (396)

FORMULA	A.H.I.*		
	$X = OCH_2CH_2N(C_2H_5)_2$	$X = NHCH_2CH_2N(C_2H_5)_2$	$X = \overset{C_2H_5}{N}CH_2CH_2N(C_2H_5)_2$
	1	1	4
	1	1	1
	2	2	1.5
	3	3	1

* A.H.I. = antihistamine index expressed as the number of M.L.D.'s necessary to kill an animal protected by 5 mg. of compound injected intravenously. Thus, A.H.I. = 1 indicates no activity.

Staub (397) attempted to determine the mode of action of I and II. She reported that, while I suppressed the action of other substances capable of contracting smooth muscle, such as acetylcholine and barium chloride, II diminished the action of these substances only slightly or not at all. Some correlation between the spasmolytic action of the phenolic ethers and their antihistamine effect was found, but in the *N*-phenylethylenediamines there was a considerable divergence in these activities. The action of the compounds *in vivo* was attributed to some unexplained phenomenon by virtue of which only a part of the injected histamine reached the lungs of treated animals. No appreciable effect on histaminase and no pronounced evidence of a chemical reaction between II and histamine could be demonstrated.

Numerous investigations have verified the antihistamine activity of I and II (71, 353, 354, 434), but there appears to be no general agreement as to the specificity of these antagonists. Both compounds have been reported to be ineffective or non-specific in reducing the gastric secretion in dogs following histamine stimulation (44, 53, 154, 238).

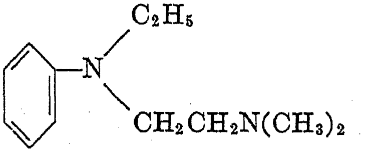
TABLE 10

Relative antihistamine activity of N-phenylethylenediamines on isolated intestine of guinea pigs (156)

COMPOUND	AMOUNT*	CONCENTRATION	RELATIVE ACTIVITY
	γ/10 cc.	M	
II	15	1.5×10^{-5}	0.1
III	1.5	1.5×10^{-6}	1
IV	0.1	1×10^{-7}	15

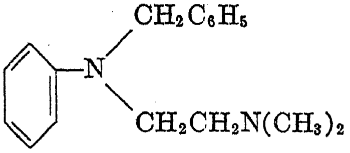
* Amount of antagonist required to counteract 90–100 per cent of the action of 2–5 × 10^{-8} *M* histamine.

Following up the work of Staub and Bovet, Halpern (156) carried out an extensive study of the *N*-phenylethylenediamine derivatives III and IV. Both of these compounds appeared to be much more active as histamine antagonists than the *N'*-diethyl derivative (II), as shown in table 10.



III

N-Phenyl-*N*-ethyl-*N'*-dimethylethylenediamine



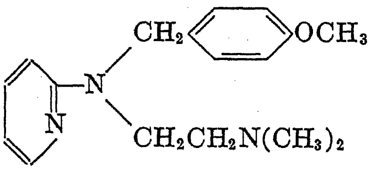
IV

N-Phenyl-*N*-benzyl-*N'*-dimethylethylenediamine

Halpern (156) studied the effect of III and IV on bronchial spasms in the intact guinea pig by employing histamine aerosols. He found the same order of activity for these compounds and II as in the *in vitro* experiments. Comparing IV with atropine and epinephrine, he reported that 1 mg./kg. of IV produced an effect equivalent to 25 mg./kg. of atropine and 0.2 mg./kg. of epinephrine. On the other hand, using acetylcholine aerosols 50 mg./kg. of IV was without effect, while 0.025 mg./kg. of atropine, and the same amount of epinephrine which was effective for histamine, controlled the bronchial spasms. These results, together with numerous *in vitro* studies, appeared to establish the specific nature of the action of the histamine antagonists. Compounds III and IV showed remarkable activity in protecting guinea pigs against the toxic action of histamine, as little as 5–10 mg./kg. of IV preventing the death of animals injected with 40–50 M.L.D.'s of histamine. Similarly, in anaphylactic shock experiments the compounds protected the sensitized animals against multiple shocking doses of serum. In many cases practically all of the symptoms of shock were eliminated. Halpern concluded that the compounds neutralized the effects of histamine by a pharmacodynamic mechanism similar to the pharmacological antagonism between atropine and acetylcholine. He suggested that the normal affinity of cells for histamine was modified in some unknown fashion by the histamine antagonists.

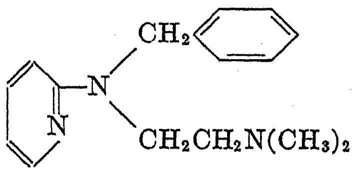
The antihistamine activity of IV was confirmed by other investigators (2, 61, 63, 284, 331). The Chauchards (63) found that IV alone produced pharmacological effects opposite to those of histamine. In different tissues the actions of the two substances individually were sometimes the reverse, but together they still neutralized each other. Thus, on cervical preganglionic fibers histamine increased chronaxia while IV caused a decrease, whereas on postganglionic fibers it was the latter which increased chronaxia and histamine decreased it. In both cases, the two substances in combination neutralized each other.

Bovet, Horclois, and Walthert (46) demonstrated that *N*-(2-pyridyl)-*N*-(*p*-methoxybenzyl)-*N*′-dimethylethylenediamine (V) also possesses a high degree of antihistamine activity in guinea pigs. A dose of 1 mg./kg. protected these animals against 75 M.L.D.'s of histamine (45 mg./kg.) and 0.1 mg./kg. was effective in preventing experimental bronchial spasms produced by histamine aerosols. *In vitro* 10^{-7} to 10^{-8} V prevented the contraction of isolated guinea pig gut produced by histamine in concentrations of 10^{-7}. The authors suggested that the action of this type of histamine antagonist was due to a competition for specific tissue receptors. In dogs, Bovet, Horclois, and Fournel (45) found that, although the results were less striking, 0.1–1.0 mg./kg. of V diminished or abolished the effects of relatively small (10–150γ/kg.) doses of histamine.



V

N-(2-Pyridyl)-*N*-(*p*-methoxybenzyl)-
N′-dimethylethylenediamine



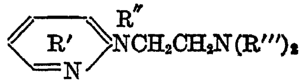
VI

N-(2-Pyridyl)-*N*-benzyl-
N′-dimethylethylenediamine

A series of closely related compounds was investigated by Mayer, Huttrer, and Scholz (284, 285), who found that *N*-(2-pyridyl)-*N*-benzyl-*N'*-dimethylethylenediamine (VI) was the most active member of the group (table 11). Secondary amines were all inactive or almost inactive. A concentration of VI one hundred times greater was required to abolish acetylcholine contractions. This result was contrasted with antispasmodics such as β-diethylaminoethyldiphenylacetate (Trasentin), which is one hundred times more active against acetylcholine than it is against histamine. With histamine aerosols in guinea pigs, VI was reported to be twice as active as IV. The same animals were also protected from anaphylactic shock by doses (of 0.1–1.0 mg./kg.) of VI.

TABLE 11

Comparative antihistamine activity of compounds of the type



COMPOUND			ACTIVITY*
R'	R''	R'''	
			γ/cc.
Pyridyl	Benzyl	Methyl	0.02
Pyridyl	Benzyl	Ethyl	>5
Pyridyl	Phenyl	Methyl	>5
Pyridyl	Phenyl	Ethyl	>10
Pyridyl	Isopropyl	Methyl	>10
Pyridyl	Propyl	Ethyl	>10
Pyridyl	β-Pyridyl	Methyl	>10
Pyridyl	α-Pyridyl	Ethyl	>10
β-Picolinyl	Benzyl	Methyl	2
α'-Picolinyl	Benzyl	Methyl	1
γ-Picolinyl	Benzyl	Methyl	0.2

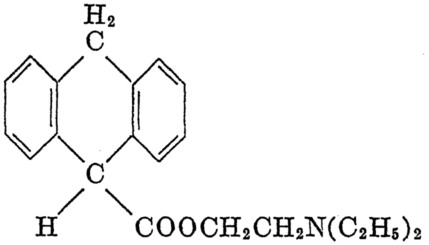
* *In vitro* activity expressed in concentrations antagonizing the action of 1 γ/cc. of histamine diphosphate on isolated intestinal strips of guinea pigs.

Additional studies by Yonkman and coworkers (336, 465, 466) indicated that VI (25–50 γ) antagonized the action of equal concentrations of histamine in the perfused lungs of guinea pigs. Histamine skin wheals produced in rabbits were also prevented by VI. In anesthetized cats VI alone produced no observed effects; histamine salivation was prevented but not mydriasis. Anaphylactic reactions in dogs were not consistently controlled by VI.

An extensive series of spasmolytic agents[11] prepared by Burtner and Cusic (54, 55) were investigated for antihistamine activity by Lehmann and Knoefel (224, 225). These compounds included esters of diphenylacetic, fluorene-9-carboxylic, 9,10-dihydroanthracene-9-carboxylic, and a number of related acids.

---

[11] Many general spasmolytic agents may fall in the category of physiological antagonists (i.e., effects due to opposite but independent actions). They are included in these instances for completeness and because the mechanism of action of most of the antihistamine agents is still largely unknown.

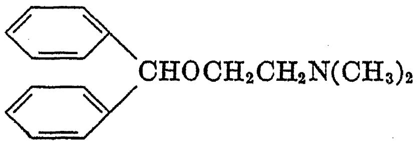


VII

β-Diethylaminoethyl 9,10-dihydroanthracene-9-carboxylate

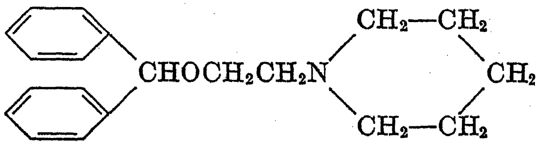
Although these derivatives also antagonized the action of acetylcholine and other spasmogenic agents, VII in particular showed a marked antihistamine effect both *in vitro* and *in vivo*. No close relationship between the antagonistic action toward histamine and acetylcholine was observed among these types of compounds. Thus, replacing the methylene group of VII with oxygen (β-diethylaminoethyl xanthene-9-carboxylate) led to a tenfold drop in antihistamine activity but a corresponding increase in acetylcholine antagonism, whereas replacement by an NH group decreased the former activity but left the latter practically unchanged. A subcutaneous dose of 50 mg./kg. of VII protected guinea pigs from 1.7 mg./kg. (3 M.L.D.'s) of histamine phosphate. Lehmann and Young (226) found that these animals were also protected against death from anaphylactic shock, but against only one fatal dose of antigen. The actions of histamine other than those on smooth muscle were not affected by VIII.

Another large group of antispasmodics (benzohydryl ethers) was studied by Loew, Kaiser, and Moore (241). These compounds were synthesized by Rieveschl and Huber (338). Employing a histamine aerosol technique similar to that used by Halpern (156), these investigators reported that many of the benzohydryl ethers were much more effective than theophylline–ethylenediamine (aminophyllin) in protecting guinea pigs from otherwise fatal doses of atomized histamine. Compared with II, VIII and IX appeared to be ap-



VIII

Benzohydryl β-dimethylaminoethyl ether



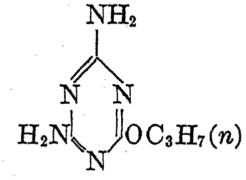
IX

Benzohydryl β-piperidinoethyl ether

proximately twice as effective. In general, compounds with longer or branched aliphatic chains were less active. The character of the nitrogen atom affected activity so that the order of effectiveness was tertiary amine > secondary > primary. Smaller groups on the nitrogen usually produced more active compounds, while replacement of the oxygen atom by NH led to inactive compounds. Loew and Kaiser (239) also found VIII and IX to be effective in alleviating experimental anaphylactic shock in guinea pigs. The potency of VIII was at least equivalent to that of II, while IX was less than one-half as active.

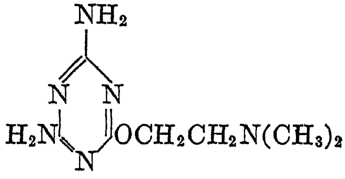
The antihistamine activity of VIII was confirmed by other investigators. Ellis (101), employing perfused guinea pig lungs, noted that not only did VIII control histamine bronchial spasms, but it also caused a significant amount of dilatation. The quantitative relationship between VIII and histamine was studied by Wells, Morris, Bull, and Dragstedt (429, 429a). These investigators found that the amount of histamine antagonized bore a constant relationship to the amount of histamine injected, and concluded that VIII might compete with histamine for its site of action.

Controulis and Banks (75) prepared a number of 2-alkoxy-4,6-diamino-*s*-triazines. These compounds were examined for antihistamine activity by Loew, Kaiser, and Anderson (240), employing the histamine aerosol method in guinea pigs (241). The most active members of this series were the propoxy derivative (X), some of the butoxy derivatives, and the thio ether corresponding to X, which were approximately four times as active as aminophyllin. XI was considerably less potent than the alkyl derivatives, while substitution on the 4- and 6-amino groups did not appear to alter activity appreciably.



X

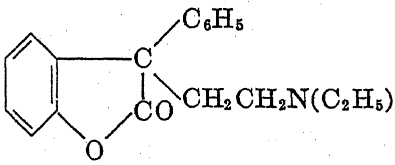
2-Propoxy-4,6-diamino-*s*-triazine



XI

2-(β-*N*,*N*-Dimethylaminoethoxy)-4,6-diamino-*s*-triazine

Richards, Everett, and Kueter (336a) investigated the antihistamine effect of XII, one of a series of benzofuranone antispasmodics. This compound was found to antagonize histamine contractions of the isolated guinea pig uterus. It also counteracted bronchial spasms induced by histamine more effectively

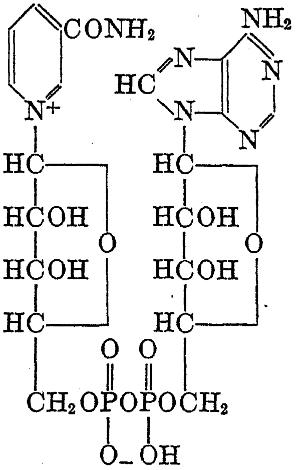


XII

3-(β-Diethylaminoethyl)-3-phenyl-2-benzofuranone than theophylline. Other studies with XII indicated that it usually reduced anaphylactic symptoms and prevented histamine shock in guinea pigs.

F. Nicotinic acid; cozymase

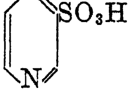
Nicotinic acid and its amide are more or less equivalent for most organisms which are unable to synthesize them (321). As a constituent part of coenzymes I and II, nicotinamide is generally recognized as an important factor in bacterial metabolism as well as animal nutrition. The coenzymes with various apoenzymes (specific proteins) function as dehydrogenases in a number of fundamental respiratory processes.



Cozymase (coenzyme I)

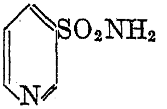
Coenzyme II differs from cozymase only in having an additional phosphoric acid residue in the molecule (104). Despite the known functions of these systems, many of the results obtained with antagonists have been inconclusive. Some of the inconsistencies may be due to the complex nature of the enzyme systems. Under the proper conditions an antagonist could presumably interfere directly or indirectly with (*a*) the synthesis of nicotinic acid or its conversion to the amide, (*b*) the production of coenzymes from either the acid or amide, (*c*) the combination of the coenzyme with a specific apoenzyme, or (*d*) the action of the holoenzyme (coenzyme + apoenzyme) on its substrate.

In 1940, McIlwain (249) reported that $M/100$ pyridine-3-sulfonic acid (I) inhibited the growth of *Proteus vulgaris* when nicotinic acid was used as a growth-promoting agent, but not when corresponding concentrations of nicotinamide



I

Pyridine-3-sulfonic acid



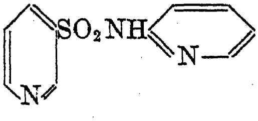
II

Pyridine-3-sulfonamide

were employed. He postulated that the action of I might be localized at the enzymatic reaction converting nicotinic acid to its amide or at a similar stage of amide formation from a more complex derivative. Growth stimulated by cozymase was even more strongly inhibited by I, suggesting that nicotinic acid was not used by *Proteus* solely for the synthesis of cozymase. With *Staph. aureus*, II ($M/100$–$M/500$) inhibited growth promoted by nicotinamide more effectively than that promoted by the acid. Inhibition of nicotinamide-stimulated growth by I was differentiated from II in that it appeared to change with time and was not directly related to the concentration of growth factor. Only temporary and incomplete effects were observed with II on *E. coli*, which does not require preformed nicotinic acid or amide. McIlwain (249) distinguished three types of growth inhibition, depending on the effect of time and changes in the ratio of growth factor and antagonist.

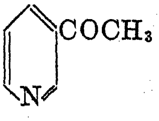
Matti, Nitti, Morel, and Lwoff (282) did not observe any growth inhibition by II ($M/80$) with their strains of proteus and coli. Employing *Proteus vulgaris* and *Streptobacterium plantarum* as test organisms, Möller and Birkofer (298) found I and II to have slight growth-promoting activity, although inhibition was sometimes produced at higher concentrations. In a careful study these investigators (298) concluded that, since I and II were bacteriostatic only in concentrations ($M/10$) at which nicotinic acid or nicotinamide also produced growth inhibition, the action under their conditions appeared to be non-specific with respect to these growth factors. They also demonstrated that ferric citrate reversed the antibacterial activity of high concentrations of II.

On the basis of the analogy between sulfanilamide and sulfapyridine, McIlwain (250) also prepared 2-(3′-pyridinesulfonamido)pyridine (III). In this case, however, III was less active as an antagonist of nicotinic acid or its amide than I or II. Auhagen (21) found that 3-acetylpyridine failed to inhibit the growth of *Streptobacterium plantarum* except at high concentrations where the action could not be prevented by nicotinic acid.



III

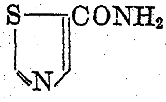
2-(3′-Pyridinesulfonamido)pyridine



IV

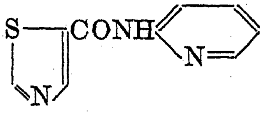
3-Acetylpyridine

Erlenmeyer, Bloch, Kiefer, and Würgler (111, 112) investigated a series of compounds structurally related to nicotinic acid. When *Proteus vulgaris* was the test organism, Erlenmeyer and Würgler (112) confirmed McIlwain's results



V

5-Thiazolecarboxamide



VI

2-(5′-Thiazolecarboxamido)pyridine

(249) with I. They also reported that V reversed the growth inhibition produced by I. On the other hand, with *Staph. aureus* V was bacteriostatic at $M/1000$ and the effect was reversed by $M/25{,}000{,}000$ nicotinamide (111). With this same organism I, in combination with nicotinic acid, produced a slight growth promotion while alone it appeared to have no effect. The bacteriostatic action of $M/5000$ II was prevented by nicotinamide at a concentration of $M/12{,}500{,}000$. III appeared to be a growth factor alone at $M/1000$, while VI produced growth inhibition which could be reversed with nicotinic acid.

Euler (113) pointed out that by working with the isolated enzyme systems it might be possible to decide which enzyme was being inhibited. The system substrate–apoenzyme–coenzyme could be inhibited either through the substrate or the coenzyme. An antagonist might also block the active group of the coenzyme or inactivate or occupy groups which confer specificity to the apoenzyme. Using glucose and lactic dehydrogenases, Euler investigated a number of substances and found that many of them inhibited these enzymes. Nicotinic acid produced about the same degree of inhibition as pyridine-3-sulfonic, benzenesulfonic, benzoic, or *p*-aminobenzoic acid. Salicylic acid, adenosine, and yeast tetranucleotide were somewhat more effective inhibitors.

In a subsequent paper Euler and Ahlström (115) reported a more complete study of salicylate [S], since it had been demonstrated that the inhibiting effect of this substance could be reversed by cozymase. Assuming that the inhibition depended on a displacement of cozymase on the apoenzyme, the authors set up the following displacement equilibrium:

$$K = \frac{[\text{cozymase–apoenzyme}][\text{NaS}]}{[\text{S–apoenzyme}][\text{cozymase}]}$$

At 50 per cent inhibition the concentration of both molecular complexes should be equal, so that,

$$K = \frac{[\text{S}]}{[\text{cozymase}]}$$

With 100 mg. of glucose and 400 mg. of apoenzyme the following data were obtained:

γ-COZYMASE	MILLIMOLES OF COZYMASE	SALICYLATE	MILLIMOLES OF SALICYLATE	K
10	0.00503	1600	3.33	662
30	0.01515	5000	10.40	686
43	0.02163	7000	14.56	673
50	0.02515	8400	17.60	692

Varying the concentration of apoenzyme had practically no effect on the degree of inhibition. Moreover, preliminary incubation of cozymase and apoenzyme for 2 hr. had no effect on the per cent inhibition. Euler and Ahlström considered that the equilibrium constant might also be explained by the reaction of salicylate with another group in the apoenzyme. They concluded that, since so many different substances produced an inhibition of the same system, because succinic dehydrogenase which does not require cozymase was inhibited by some of the same compounds, and since salicylate inhibited both cocarboxylase and cozymase, it was improbable that its action depended on a specific displacement of the coenzyme.

In mice, Woolley and White (459) were unable to produce a nicotinic acid deficiency by feeding pyridine-3-sulfonic acid. They suggested that the lack of effect might be due to the fact that mice do not require an external source of nicotinic acid. It had been shown earlier that dogs deficient in nicotinic acid were killed by the sulfonic acid while normal dogs were apparently not harmed (456). 3-Acetylpyridine (IV) produced a similar effect. Later, Woolley (452) reported that typical signs of nicotinic acid deficiency in mice were produced by IV. The signs of the disease were prevented by an excess of nicotinic acid or nicotinamide in the rations. With several species of bacteria, no specific growth inhibition with IV was observed, confirming the results obtained by Auhagen (21).

About the time that Woods and Fildes (122, 443) described the relationship of PABA to sulfonamides, West and Coburn (430) reported that cozymase interfered with the antibacterial action of sulfapyridine, while nicotinic acid did not. Since autoclaved cozymase was used as a control, it seemed unlikely that this effect could be due to contamination with PABA. The authors called attention to a structural relationship between sulfapyridine and nicotinic acid, and suggested that the two substances might compete for the same position in the coenzyme molecule. Dorfman, Rice, Koser, and Saunders (91) studied the effect of sulfapyridine on the respiration of dysentery bacilli. When glucose was used as substrate, it was found that respiration stimulated by nicotinamide was inhibited by sulfapyridine. The order of addition of these substances was important, a greater inhibition resulting from the addition of sulfapyridine first, followed by a period of incubation before the nicotinamide was added. Similar results were observed with cozymase, and the authors suggested that the action of sulfapyridine on microörganisms might be related to the rôle of nicotinamide in their metabolism.

Numerous investigators (90, 183, 298, 371, 390, 404, 411, 442) have examined these relationships to bacterial growth and respiration with variable results. Anderson, Pilgrim, and Elvehjem (10), working with enzyme systems in which coenzyme I was necessary for maximum activity, found no inhibition with sulfapyridine or sulfathiazole. They concluded that these sulfonamides did not interfere directly with the functioning of cozymase in yeast fermentation or in several systems in normal rat liver, and suggested that the available evidence indicated the possibility that the sulfonamides might inhibit the synthesis of coenzymes.

G. *Pantothenic acid*

Although pantothenic acid is widely distributed in nature, its physiological functions are largely unknown at present. Available evidence suggests the possibility that it may be associated with carbohydrate metabolism (436). It seems likely that the vitamin is necessary for the normal functioning of practically all living matter (351). β-Alanine and α-hydroxy-β,β-dimethyl-γ-butyrolactone (pantolactone) or the corresponding acid (pantoic acid) are generally regarded as the precursors in the biological synthesis of pantothenic acid. Many closely related derivatives have been tested for vitamin activity, but at most they have very slight physiological action or are incomplete substitutes for natural d(+)-pantothenic acid (436).

$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2COOH$

Pantothenic acid

$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2SO_3H$

Pantoyltaurine

Snell (380) and Kuhn, Wieland, and Möller (210) first described the taurine analogue of pantothenic acid, for which the name pantoyltaurine was suggested by McIlwain (254). With a strain of *Lactobacillus arabinosus* which requires preformed pantothenate, Snell found that pantoyltaurine was bacteriostatic, and that over a wide range of concentrations, growth was proportional to the relative amounts of pantoyltaurine and pantothenic acid. A strain of yeast stimulated by pantothenic acid was inhibited by pantoyltaurine, but growth of the same yeast stimulated by β-alanine was not reduced by the antagonist or by taurine. A number of other organisms requiring preformed pantothenic acid were susceptible to pantoyltaurine in varying degrees, and growth could be restored by additional pantothenic acid. In these cases the antibacterial action of the antagonist appeared to be produced entirely by its effect in screening the essential vitamin away from its site of action. However, organisms which synthesized their own pantothenic acid were not susceptible to pantoyltaurine. It was suggested that differences in cellular permeability or pantothenic acid metabolism might explain the lack of effect on these organisms. Since only d(+)-pantothenic acid acts as a growth factor for *L. arabinosus*, Snell investigated the optical isomers of pantoyltaurine. The d(−)- and l(+)-α-hydroxy-β,β-dimethyl-γ-butyrolactones were condensed with the sodium salt of taurine by heating for 5 hr. at 120°C. The pantoyltaurine from the d(−)-lactone proved to be about ten times as active as a growth inhibitor, and because of the racemization which undoubtedly occurred at the high temperature, all the antibacterial action was considered to be due to this isomer.

Under milder conditions (60 hr. standing at room temperature, followed by chromatographing and conversion to the quinine salt) Kuhn, Wieland, and Möller (210) obtained a d(+)-pantoyltaurine which was 32 times as active as the corresponding l(−)-isomer. Since their lactone intermediates were not optically pure, these investigators also concluded that all the bacteriostatic activity was probably due to the d(+)-form of pantoyltaurine. With *Streptobacterium plantarum* Kuhn *et al.* found that growth inhibited by pantoyltaurine was completely restored by pantothenate and partially by β-alanine, or better, β-alanine plus pantolactone, although neither of the latter could replace pantothenic acid as a growth factor for the organism. The bacteriostatic action decreased with time, and it was suggested that either the antagonist was gradually split or the presence of pantoyltaurine might stimulate the organisms to synthesize pantothenate. No other vitamins or growth factors tested were found to reverse the action of pantoyltaurine, thus demonstrating the specific nature cf the growth inhibition. Assuming that metabolite and antagonist compete for the same apoenzyme in the cell, the inhibition constant at 50 per cent growth inhibition and from this the dissociation constant of the apoenzyme–pantothenate and apoenzyme–pantoyltaurine complexes were calculated as indicated:

$$K_p = \frac{[\mathrm{P}] \times [\mathrm{Ap}]}{[\mathrm{PAp}]} = 2 \times 10^{-8}$$

$$K_t = \frac{[\mathrm{T}] \times [\mathrm{Ap}]}{[\mathrm{TAp}]} = 7.6 \times 10^{-6}$$

where [P] = concentration of pantothenate, [Ap] = concentration of apoenzyme, and [T] = concentration of pantoyltaurine. These values were of the same order of magnitude as the dissociation constants for previously determined cell-free enzyme systems such as the old yellow enzyme (3.3×10^{-5}) and α-amino acid oxidase (2.5×10^{-7}).

Teague and Williams (410) reported that the fermentation by deficient yeast cells, which was accelerated by pantothenate, was not affected by pantoyltaurine.

McIlwain (254, 255) found that racemic pantoyltaurine inhibited the growth of streptococci, pneumococci, and some strains of diphtheria bacilli, but not coli, staphylococci, or *Proteus morganii*, even though the last-named organism required preformed pantothenic acid. With streptococci and pneumococci neither β-alanine nor pantoic acid interfered with the antibacterial action of pantoyltaurine, but in most cases β-alanine as well as pantothenate acted as reversing agents with susceptible strains of diphtheria bacilli. Blood serum and other natural fluids also reversed the bacteriostatic effect of pantoyltaurine, and it was shown that this action was directly related to the pantothenic acid content of these fluids. From the quantitative relationships between pantothenate and pantoyltaurine which he obtained, McIlwain considered that the results were typical of the competitive inhibition of an enzyme. He found that for streptococci the inhibition ratio was approximately 500:1, while for pneumococci it was about 1000:1. Allowing for the probable inactivity of the *l*(−)-isomer (210, 380), the ratios were 250:1 and 500:1, respectively. The ratios for several other organisms were determined to be considerably higher. On the basis of the blood pantothenate levels and the inhibition ratios, McIlwain concluded that it should be possible to obtain an antistreptococcal level of pantoyltaurine in experimental animals.

A study of the therapeutic effects of pantoyltaurine in experimental streptococcic infections was carried out by McIlwain and Hawking (264). They found that this substance was rapidly excreted even when large doses were injected. In mice pantoyltaurine was ineffective as a chemotherapeutic agent. This result was shown to be due to the amount of pantothenic acid present in mouse blood. Rats, in which the blood pantothenate is lower, were completely protected by large frequent doses of pantoyltaurine. The simultaneous administration of pantothenic acid reversed the action of the antagonist, as it did *in vitro*. As the authors pointed out, the importance of these studies rests on the demonstration that, provided allowance is made for the concentration of the natural reversing agents present in blood and tissues, antagonists designed to inhibit specific essential metabolites can function *in vivo* as well as *in vitro*.

Attempts have been made to produce a pantothenic acid deficiency in animals by means of pantoyltaurine. Snell, Chan, Spiridanoff, Way, and Leake (383) reported obtaining symptoms of such a deficiency in mice by long-continued oral administration of pantoyltaurine. However, Woolley and White (459) and Unna (415) were unable to confirm these results.

In addition to pantoyltaurine, the following analogues of pantothenic acid were prepared by Barnett and Robinson (27):

$$\mathrm{HOCH_2(CH_2)_2CONH(CH_2)_2COOH}$$

I

Bisnordeoxypantothenic acid

$$\mathrm{CH_3CHOH(CH_2)_2CONH(CH_2)_2COOH}$$

II

Isonordeoxypantothenic acid

$$\mathrm{HOCH_2C(CH_3)_2CHOHCH_2CONH(CH_2)_2COOH}$$

III

Homopantothenic acid

$$\mathrm{HOCH_2C(CH_3)_2CH_2CONH(CH_2)_2COOH}$$

IV

Deoxypantothenic acid

$$\mathrm{HOCH_2C(CH_3)_2CH{=}CHCONH(CH_2)_2COOH}$$

V

Dehydrohomopantothenic acid

$$\mathrm{HOCH_2C(CH_3)_2CHOHCONH(CH_2)_2SO_2NH_2}$$

VI

Pantoyltauramide

$$\mathrm{HOCH_2C(CH_3)_2CH_2CHOHCONHCH_2CH_2SO_3H}$$

VII

Homopantoyltaurine

These and several other derivatives synthesized by condensing pantolactone with various amino acids had very little growth factor activity.

The compounds prepared by Barnett and Robinson were studied as pantothenate antagonists by McIlwain (255). He found that the other taurine derivatives (VI and VII) behaved like pantoyltaurine, although they were in general less inhibitory to growth. The antistreptococcal index was 2000 for the tauramide derivative (VI) and 20,000 for VII, as compared with 500 for pantoyltaurine. With other susceptible organisms the same order of effectiveness was observed. Compounds I and II were slightly bacteriostatic for streptococci, and more so for coli and staphylococci, but pantothenic acid did not reverse the effect of these two substances. The other derivatives were inactive as antagonists. To account for the observation that the taurine derivatives in general only inhibited the growth of organisms requiring preformed pantothenic acid, McIlwain proposed the following possible explanations: (*a*) Destruction of the inhibitor during growth; (*b*) production of large amounts of pantothenic acid; or (*c*) other inherent differences in enzyme make-up or permeability. It was possible to rule out both (*a*) and (*b*) in the case of *E. coli*, but the factors in (*c*) were more difficult to assess. In relation to (*b*) McIlwain demonstrated that pantoyltaurine did not stimulate coli to produce more pantothenate. He also suggested that pantothenic acid produced *in situ* might be a more effective reversing agent than when supplied in the culture medium.

By successive subculturing in increasing concentrations of pantoyltaurine, McIlwain (259) obtained resistant strains of streptococci and diphtheria bacilli. Fast strains of streptococci were found to be no more resistant to sulfanilamides than the parent organisms and, conversely, sulfanilamide-fast bacteria were just as susceptible to pantoyltaurine as the parent strains. The pantoyltaurine-resistant streptococci retained a need for pantothenic acid, whereas the requirements of diphtheria bacilli changed, so that strains originally requiring pantothenate were able to grow with β-alanine and produced pantothenate when they became fast to pantoyltaurine. The resistant streptococci apparently neither produced more pantothenate nor destroyed the pantoyltaurine. Resistant diphtheria bacilli were also obtained by "training" without the use of pantoyltaurine. Organisms "trained" to utilize β-alanine efficiently were more resistant to pantoyltaurine, while those which became dependent on pantothenic acid were more susceptible.

McIlwain (259) also found that the growth of *Proteus morganii*, which was resistant to pantoyltaurine, was inhibited by a combination of this antagonist and salicylate [*cf.* Ivanovics (186)]. Concentrations of these substances which alone were without effect produced complete bacteriostasis in combination. A similar synergistic action was observed with both the normal and resistant streptococci. Pantothenate reversed the combined bacteriostatic action in all cases.

In a later investigation McIlwain (260) reported that two strains of *Proteus morganii* were susceptible to pantoyltaurine, and visible growth was prevented by ratios of antagonist to pantothenate of 2×10^5. He also studied the mode of action of pantoyltaurine by determining its effect on the gas production of streptococci under anaerobic conditions. Although the effects of the antagonist could be reversed by pantothenate, they appeared to be too small to account for the marked effect of pantoyltaurine on the growth of the organism. McIlwain concluded "that pantothenate is of importance to the organism because of the products to which it normally leads, whose presence in adequate concentrations is necessary for normal growth. When pantoyltaurine affects growth, it is believed to lower the rate of formation of these products to such an extent that they limit the rate of growth of the organism."

McIlwain and Hughes (265) reported that pantoyltaurine prevented the disappearance of pantothenate from streptococcal cultures, which normally occurred in its absence. This metabolism of pantothenate was associated with carbohydrate metabolism but was independent of growth and oxygen supply. Other substances which prevented glycolysis reduced pantothenate metabolism, but pantoyltaurine stopped this metabolism without interfering with glycolysis. Streptococci were shown by McIlwain (262) to inactivate pantothenic acid (i.e., destroy its growth-promoting properties) at the rate of about 2 millimicromoles per milligram of dry weight per hour. Pantoyltaurine was also inactivated by these organisms, although at a slower rate. Of various bacterial species studied, five which synthesized pantothenic acid were insensitive to pantoyltaurine, while six requiring the preformed metabolite for growth were sensitive in varying degrees to the antagonist. However, there did not appear to be any direct relationship between the rate at which the organisms destroyed pantothenic acid and their sensitivity to pantoyltaurine.

After determining the inhibition ratios of a group of pantothenic acid antagonists and related compounds (25, 26, 274), McIlwain and Hughes (266) showed that these ratios could be correlated with the effectiveness of the antagonists in preventing pantothenate destruction. Similarly, the relative antibacterial activity of pantoyltaurine against various microörganisms was directly related to its protective action. Analogous compounds which were not reversed or were inactive as growth inhibitors did not protect pantothenic acid from inactivation by the organisms. These results suggested that the two phenomena, metabolism and growth, were closely related, and that the effect of antagonists on growth might be associated with their inhibition of pantothenate inactivation. McIlwain (263) found that, while there was a latent period in the growth-inhibitory action of pantoyltaurine, pantothenate inactivation was affected immediately. However, he did not consider that this observation was inconsistent with a causal relation between the two inhibitions, because the latter process was probably carried out by the organisms in considerable excess of their minimal needs.

McIlwain (263a) also attempted to demonstrate a displacement of pantothenate in bacterial cells by pantoyltaurine. No liberation of firmly bound pantothenate was observed in the presence of excess pantoyltaurine. Larger quantities of the metabolite which were loosely associated with the organism (β-hemolytic streptococci) were released into saline solutions, but pantoyltaurine did not increase the quantity of loosely bound pantothenate released. These results were taken as additional evidence that the antagonist acts by preventing the binding of pantothenate in functioning form by susceptible organisms, rather than by the displacement of firmly bound metabolite.

While studying the biological action of α-methylpantothenic acid, Pollack (324) found that high concentrations of the compound repressed the growth-promoting action of pantothenic acid on *Lactobacillus casei.* This repression increased with concentration only up to a point, beyond which additional amounts led to slightly increased growth. When it occurred, the growth-inhibiting action of VIII could be reversed by the addition of excess pantothenic acid. With a yeast strain the α-methyl derivative produced some growth stimulation, but this effect could not be attributed to hydrolysis of the compound followed by utilization of the pantoic acid. Under certain conditions Pollack found that α-methyl-β-alanine also appeared to inhibit the growth of yeast to a slight degree.

$$\mathrm{HOCH_2C(CH_3)_2CHOHCONHCH_2CH(CH_3)COOH}$$

VIII

α-Methylpantothenic acid

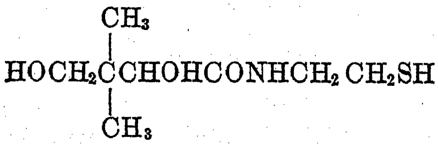
$$\mathrm{HOCH_2C(CH_3)_2CHOHCONHCH(CH_3)CH_2COOH}$$

IX

β-Methylpantothenic acid

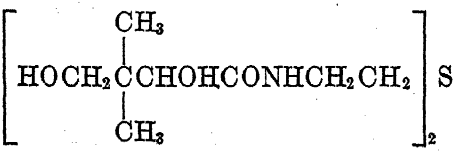
Nielsen, Hartelius, and Johansen (308) found that β-methylpantothenic acid (426) inhibited the growth of *Streptobacterium plantarum*, the ratio of antagonist to pantothenic acid being about 5000:1. With ratios as low as 200:1, growth was inhibited approximately 50 per cent. The effect of IX on the growth of yeast cells was apparent only in the presence of higher ratios (20,000–50,000:1), although the respiration of these cells was inhibited at somewhat lower levels. Shive and Snell (375) also examined the effect of crude VIII and IX on several microörganisms. In general, racemic IX appeared to be a somewhat more effective antagonist, and with some organisms (e.g., *Leuconostic mesenteroides* P-60) VIII did not produce complete inhibition of growth. In confirmation of Pollack's work (324), it was found that VIII inhibited growth only to the level corresponding to its own stimulatory effect.

Barnett (25) prepared several analogues of pantoyltaurine from β-mercaptoethylamine, β,β′-diaminodiethyl sulfide, and the corresponding disulfide. From the sulfide, the sulfoxide and sulfone were obtained. The *N*-pantoyl derivatives of these compounds, e.g.,



$$\mathrm{HOCH_2\overset{CH_3}{\underset{CH_3}{C}}CHOHCONHCH_2\,CH_2SH}$$

N-Pantoyl-β-mercaptoethylamine



$$\left[\mathrm{HOCH_2\overset{CH_3}{\underset{CH_3}{C}}CHOHCONHCH_2CH_2}\right]_2\mathrm{S}$$

β,β′-Di(*N*-pantoylaminoethyl) sulfide

inhibited the growth of *Lactobacillus arabinosus.* The mercapto and disulfide compounds were reported to be as active as pantoyltaurine, while the sulfide, sulfoxide, and sulfone were less effective. All of the pantoyl derivatives were reversed by pantothenic acid. *In vivo* tests in rats infected with streptococci apparently did not indicate that these analogues were as effective as pantoyltaurine, although no experimental details were reported.

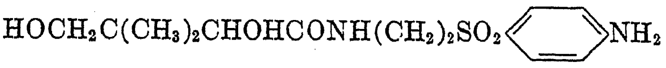
Substitution of phenyl for methyl groups, or the introduction of a phenyl substituent alpha to the sulfonic acid group in pantoyltaurine was reported by Barnett, Dupré, Holloway, and Robinson (26) to yield inactive compounds. An attempt was also made to decrease the rate of excretion by esterification of the α-hydroxyl with *p*-toluenesulfonyl chloride, in the hope that this derivative might gradually break down *in vivo* to pantoyltaurine. However, the compound was inactive when tested against experimental streptococci infections in rats.

Madinaveitia, Martin, Rose, and Swain (274) prepared and studied a series of pantamide derivatives including the amide itself, the hydrazide (X), and dialkylamino-alkylamino, phenylalanine, hexahydroanthranilic acid and other derivatives as well as several β-aminoethyl phenyl sulfones such as XI. A majority of these compounds acted as pantothenic acid antagonists when *L. casei* ϵ and streptococci were the test organisms. X was the most potent antagonist in the presence of low concentrations of pantothenic acid. At higher concentrations of the metabolite, however, this derivative and others, including XI and the corresponding *p*-methyl and *p*-methoxy derivatives, were similar to pantoyltaurine in activity. The antibacterial action of compound XI was reversed by pantothenic acid (2.5γ/cc.) but not by PABA (1/1000), indicating that antagonism of the latter metabolite was not contributing to the antibacterial action. *In vivo* XI showed a slight chemotherapeutic effect in experimental streptococcal infections in rats.

$$\mathrm{HOCH_2C(CH_3)_2CHOHCONHNH_2}$$

X

dl-Panthydrazide



XI

dl-β-(*N*-Pantoylaminoethyl) *p*-aminophenyl sulfone

Several alcohols and related compounds were investigated by Snell and Shive (375, 386), who showed that pantothenyl alcohol (386) (XII) and other alcohols

$$\mathrm{HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CH_2OH}$$

XII

Pantothenyl alcohol

$$\mathrm{HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2OH}$$

XIII

dl-*N*-Pantoylethanolamine

$$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CH(CH_3)OH$$

XIV

N-Pantoyl-4-amino-2-butanol

$$HOCH_2C(CH_3)_2CHOHCONHCH_2CHOHCOOH$$

XV

N-Pantoylisoserine

(XIII, XIV, and XV) inhibited the growth of several microörganisms. These derivatives were not obtained in pure form, but it was found (386) that XII prepared from *d*(−)-pantolactone was twice as active as the product from *dl*-lactone and about ten times more effective than XII from *l*(+)-pantolactone. With *Leuconostic mesenteroides* P-60 growth stimulated by pantothenic acid was inhibited by XII over a wide range of concentrations. The competitive nature of this action was demonstrated by the constant ratio (300:1). A physical mixture of 3-amino-1-propanol and *d*(−)-pantolactone did not show any antagonistic activity. No bacteriostatic effect was observed with organisms not requiring pantothenate, such as *Staph. aureus* and *E. coli.* When XII and pantoyltaurine were compared, a marked variation in the susceptibility of different organisms to these two antagonists was observed (table 12).

TABLE 12

Comparative susceptibility of various organisms to inhibition by pantothenyl alcohol and pantoyltaurine (386)

ORGANISM	ANTIBACTERIAL INDEX*	
	Pantothenyl alcohol	Pantoyltaurine
Leuconostic mesenteroides P60	300	200,000
Lactobacillus acidophilus VT	5,000	5,000
Lactobacillus arabinosus	5,000	1,000
Lactobacillus casei	10,000	10,000
Lactobacillus fermentum	100,000	200,000
Streptococcus fecalis R	50,000	30,000
Streptococcus lactis 125	1,000	
Saccharomyces cerevisiae FB	30,000	10,000
Staphylococcus aureus	Not inhibited†	
Escherichia coli	Not inhibited†	Not inhibited

* Ratio antagonist: metabolite for complete inhibition of growth.

† Maximum concentration tested, 3 mg./cc.

Woolley and Collyer (454) synthesized and studied phenyl pantothenone, a

$$HOCH_2C(CH_3)_2CHOHCONH(CH_2)_2COC_6H_5$$

Phenyl pantothenone

ketone analogue of pantothenic acid. This compound inhibited the growth of a number of microörganisms. As indicated in table 13, higher concentrations of pantothenic acid reversed the growth inhibition in a number of instances. However, in two cases[12] the antagonism of organisms not requiring preformed pantothenate was not prevented by the metabolite. With *Staph. aureus,* pantothenic acid was only stimulatory, and the antibacterial effect of phenyl pantothenone was reversible. A sample of what appeared to be impure methyl pantothenone was also studied by Woolley and Collyer. The growth-inhibitory effect of this product was not prevented by pantothenic acid when either *L. casei* or *S. cerevisiae* was the test organism.

Shive and Snell (375a) prepared a group of *dl-N*-pantoylalkylamines as well as the corresponding derivatives of several substituted alkylamines. All of these compounds inhibited the growth of several microörganisms which required preformed pantothenic acid. The different antagonists varied considerably in their inhibitory effect on the same organism, and the relative activity of different compounds, even in a homologous series, changed markedly with different organisms. In general, the inhibition ratios were of the same order of magnitude as those for pantothenyl alcohol.

TABLE 13

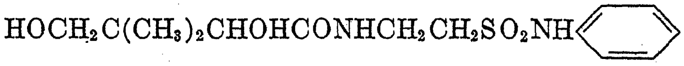
Amounts of phenylpantothenone required to reduce growth of various microörganisms (454)*

ORGANISM	ANTAGONIST	PANTOTHENIC ACID REVERSAL	PANTOTHENIC ACID REQUIREMENT
	γ/cc.		
Lactobacillus casei	54	+	Required
Lactobacillus arabinosus	180	+	Required
Streptococcus hemolyticus (Group B)	60	+	Required
Escherichia coli†	2000	−	Not required
Staphylococcus aureus	140	+	Not required but slightly stimulatory
Saccharomyces cerevisiae	33	−	Required but replaceable by β-alanine
Endomyces vernalis	39	−	Not required

* Half-maximum growth in the presence of 0.04 γ/cc. of pantothenic acid.

† Complete inhibition of growth not obtained with this organism.

A large series of pantoyltauramides was prepared by Winterbottom, Clapp, Miller, English, and Roblin (437). Included in this series were aliphatic aromatic and heterocyclic amine derivatives, many of which possessed a high degree of antibacterial activity. White, Lee, Jackson, Himes, and Alverson (433) found that the inhibition ratio for the most active members, e.g., pantoyltaura-



Pantoyltauranilide

---

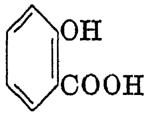
[12] The effect on *E. coli,* because of the relatively high concentrations required, may not be specific. In general, specific antagonism in the absence of reversal can only be deduced by analogy.

mido-4-chlorobenzene and pantoyltauramido-3,5-dibromobenzene, was approximately 25:1 for the $d(+)$-isomers. Several strains of streptococci and pneumococci were susceptible to these derivatives *in vitro,* but a number of other organisms tested were resistant to high concentrations (128 mg./100 cc.). Most of the resistant organisms presumably did not require preformed pantothenic acid. With a group A streptococcus in a complex medium containing 0.02 mg./100 cc. of pantothenic acid, White *et al.* obtained the following results for $d(+)$-pantoyltauranilide:

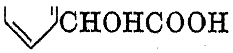
PANTOTHENIC ACID		M.E.C.	INHIBITION RATIO
Added	Total		
mg./100 cc.	*mg./100 cc.*	*mg./100 cc.*	
	0.02	1	50
0.3	0.32	16	50
1.5	1.52	64	42
6.3	6.32	128	20

The M.E.C. represented the minimum effective concentration required to inhibit growth completely for 48 hr. *In vivo,* mice were protected from experimental streptococcal infections by single oral doses of about 0.5 g./kg. of the halogen-substituted anilides. These results contrasted sharply with the activity of pantoyltaurine, which was ineffective orally and only showed chemotherapeutic activity in rats when large frequent doses were administered by injection (264).

While a majority of the antagonists probably interfere with the utilization of pantothenic acid, substances which may interfere with its synthesis have also been described. Ivanovics (185, 186) found that, in addition to a non-specific protein-denaturing action at high concentrations (0.1–0.25 *M*), small amounts of salicylic acid appeared to prevent the synthesis of pantothenate. Acetyl and phenyl salicylates were also effective, but thiosalicylic acid and salicylamide were not. As in the case described by Fildes (124), no constant ratio between salicylate and pantothenate was observed. With staphylococci, pantolactone reversed the growth inhibition of salicylate but it was much less efficient in this respect than pantothenic acid, while β-alanine had no reversing action. *Proteus morganii,* requiring preformed pantothenate, was unaffected except by high concentrations of salicylic acid. These observations led Ivanovics to conclude that the antagonism occurred at the synthesis stage, probably in the production of pantoic acid or its conversion to pantothenic acid. In a simple medium the growth of *E. coli* was inhibited by 0.00002 *M* salicylate. The presence of protein hydrolysates



Salicylic acid



Mandelic acid

almost completely eliminated this antibacterial action. Ivanovics showed that a mixture of amino acids produced the same result. Valine alone was the most efficient but this and other amino acids, including methionine, lysine, leucine, and isoleucine in combination, were still more active in reversing salicylate. Coli produced eight times as much pantothenate in a casein hydrolysate medium, and it was concluded that some of the amino acids might be involved in the synthesis of pantolactone.

Stansly, Schlosser, and Alverson (394, 395) confirmed Ivanovics' observations with *E. coli* and demonstrated that pantoate ion is approximately nine times more active than pantolactone in reversing the antibacterial action of salicylate. Pantoyltaurine, presumably because it is partially hydrolyzed to pantoic acid, was also shown to prevent the bacteriostatic effect of salicylic acid (394). The formation of pantoic acid or of pantolactone was suggested as a possible explanation for the ineffectiveness of pantoyltaurine as an antibacterial agent for microorganisms which can utilize these intermediates for the synthesis of pantothenic acid.

Pérault and Greib (318) reported that, like salicylic acid, the antibacterial action of mandelic acid in concentrations of 1 per cent or less was reversed by pantothenic acid ($M/10^6$). The activity of higher concentrations of mandelic acid was only slightly affected by pantothenic acid ($M/1000$), while PABA had no effect on the bacteriostatic activity. However, with three strains of *E. coli* which were susceptible to mandelic acid in concentrations of less than 1 per cent, White (432) was unable to demonstrate any reversal with pantothenic acid.

Nielsen (306) observed that the growth-stimulating action of β-alanine

$H_2NCH_2CH_2COOH$	$H_2NCH(CH_3)CH_2COOH$	$H_2NCH_2CH_2SO_3H$
β-Alanine	β-Aminobutyric acid	Taurine

on yeast was antagonized by β-aminobutyric acid. In the absence of β-alanine this acid had neither growth-promoting nor growth-inhibiting action. Taurine, β-hydroxypropionic acid, and β-alanylglycine were inert. The following results were obtained by Nielsen and Johansen (309):

β-AMINOBUTYRIC ACID	MILLIGRAMS OF DRY YEAST PER 100 CC.		
	No β-alanine	β-Alanine (2 mg./100 cc.)	*dl*-Sodium pantothenate (1 mg./100 cc.)
mg./100 cc.			
20	11.4	12.2	26.6
10	11.6	15.6	26.2
6	11.2	17.6	26.8
2	11.8	25.8	26.8
0	11.8	34.8	26.0

These investigators concluded that β-aminobutyric acid interfered with the synthesis of pantothenic acid from β-alanine by yeast cells. They found isoserine, but not *N*-methyl-β-alanine, to have a similar action and suggested that the antagonists might actually be converted to inert compounds containing β-aminobutyric acid or isoserine instead of β-alanine.

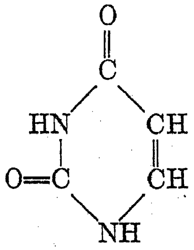
The effect of β-aminobutyric acid on the respiration of yeast was studied by Hartelius (163). His results were essentially the same as those obtained by Nielsen and Johansen (306, 309) in their growth studies. The inhibitory effect of β-aminobutyric acid on oxygen consumption was prevented by β-alanine or sodium pantothenate. Respiration stimulated by β-alanine was completely repressed by a ratio of approximately 1000:1, but the minimum effective concentration of pantothenate prevented the action of varying amounts of the antagonist. These observations also suggested that β-aminobutyric acid interfered with the synthesis rather than with the utilization of pantothenic acid.

Sarett and Cheldelin (361) investigated the effect of taurine and pantoyltaurine on 17 strains of yeast. Growth was stimulated with either β-alanine or pantothenic acid. When β-alanine (0.2–0.5 γ) stimulated growth, only one strain was inhibited 85–90 per cent with 1 mg. of taurine or 2 mg. of pantoyltaurine, all the other strains being unaffected by either antagonist. With pantothenic acid as the growth stimulant, taurine had no effect on any of the strains, but the growth of all of them was inhibited in varying degrees by pantoyltaurine.

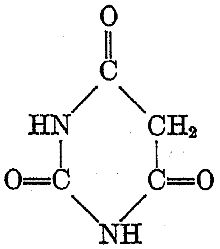
H. Purines and pyrimidines

Cell nuclei are composed largely of nucleoproteins, in which are incorporated the pyrimidine- and purine-containing nucleic acids. In addition, some of these substances in the form of nucleotides function as coenzymes or are an important part of other coenzyme molecules. The pyrimidines and purines are now recognized as growth stimulants for many microörganisms (243).

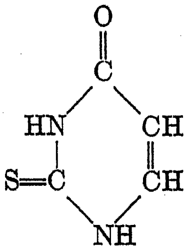
Woods (444, 445) reported that *M*/100–*M*/250 barbituric acid delayed the growth of *Staph. aureus* in a synthetic medium. The addition of uracil (*M*/5000 or less) abolished this effect and restored the normal growth rate. These observations have been confirmed by Roepke and Jones (347) with *E. coli.* They also found that 2-thiouracil produced an even greater growth inhibition than barbituric acid. In both cases uracil prevented the bacteriostatic action. The effect of 2-thiouracil was apparent only for relatively short periods of time, and high concentrations were somewhat less effective, suggesting that this substance might be gradually converted to uracil or otherwise degraded by the organisms.



Uracil

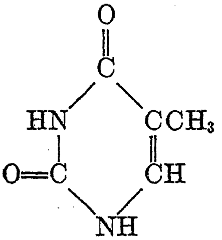


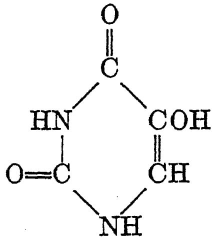
Barbituric acid

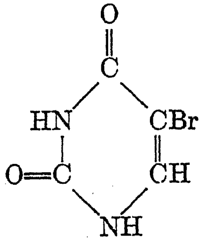


2-Thiouracil

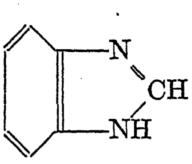
Relatively large amounts of thymine will replace folic acid as a growth stimulant for certain organisms such as *L. casei* and *Strep. fecalis* R (402). Hitchings, Falco, and Sherwood (176) noted that several 5-substituted pyrimidines related to thymine, such as uramil and isobarbituric acid, inhibited the growth of *L. casei* stimulated by either thymine or folic acid. Growth could be restored by increasing the concentration of either metabolite. Other 5-substituted derivatives, e.g., 5-bromouracil, completely inhibited thymine-stimulated growth, but had no effect, or produced a slight stimulation, when folic acid was used as the nutrient. To some extent, the reverse situation was observed with 5-nitrouracil. The authors concluded that the latter results did not fit the concept of thymine as a product of an enzyme system involving folic acid as a prosthetic group or coenzyme (402). They suggested that the two metabolites might act in some fashion as alternatives rather than as two components of an anabolic system.

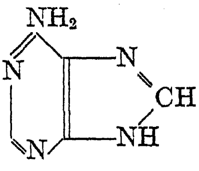

Thymine

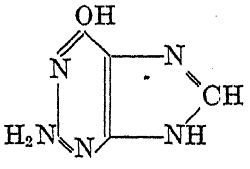

Isobarbituric acid


5-Bromouracil

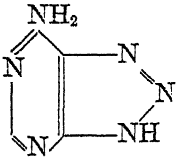
In a study of the biological effects of benzimidazole, Woolley (447) observed that the bacteriostatic action of this substance could be reversed by adenine and guanine. The latter was the more active in overcoming the effect of benzimidazole, while xanthine, hypoxanthine, and adenylic acid were ineffective in this respect. When *S. cerevisiae* was the test organism uracil also produced no reversal, but with *Strep. fecalis* R a partial reversal was observed with relatively small concentrations of this pyrimidine. All of the organisms tested, both yeasts and bacteria, were susceptible to benzimidazole. Woolley prepared 4-aminobenzimidazole, the benzene analogue of adenine, by reduction of the 4-nitro derivative. Both of these compounds inhibited growth to about the same extent as benzimidazole. 5-Aminobenzimidazole was less effective, and the 2-hydroxy derivative showed no bacteriostatic action.

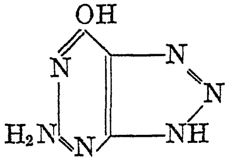

Benzimidazole

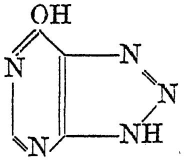

Adenine

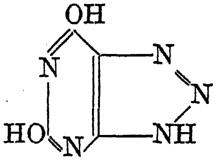

Guanine

Roblin, Lampen, English, Cole, and Vaughan (341) synthesized analogues of adenine, guanine, hypoxanthine, and xanthine, in the triazolopyrimidine series, in which a nitrogen atom replaces a carbon atom of the corresponding purine. Of these, the adenine and guanine analogues (I and II) in particular exhibited a specific antibacterial action. The results obtained with the triazolopyrimidines are summarized in table 14.


I

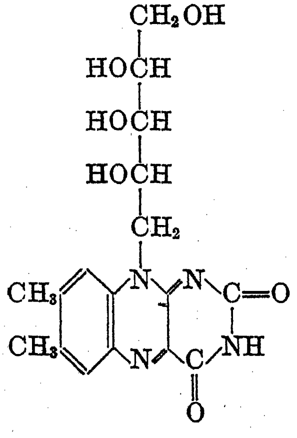

II


III


IV

When several M.E.C.'s of these compounds were employed, the growth inhibition of *E. coli* by compound I was reversed only by adenine and hypoxanthine, guanine, xanthine, uracil, cytosine, or thymine being ineffective. Similarly with II, guanine and xanthine were the only substances tested which prevented its bacteriostatic action. When barely effective amounts of the analogues were tested, the results were less specific in that all four of the purines were more or less equivalent as reversing agents. The action of the hypoxanthine analogue (III) was apparent for short periods of time only, and again all four purines prevented it. No specific antibacterial action could be demonstrated for compound IV. In combination with sulfonamides, the guanine analogue produced a synergistic effect, so that much less of each was required to produce the same degree of bacteriostasis. None of the other triazolopyrimidines displayed this effect. Sulfonamide-fast organisms were no more resistant to II than the parent strains.

I. Riboflavin



6,7-Dimethyl-9-(*d*-1′-ribityl)isoalloxazine

Riboflavin in the form of coenzymes takes part in a number of enzyme systems involved in hydrogen transfer for many important metabolic processes, including carbohydrate and amino acid metabolism. Two such coenzymes have been identified,—a riboflavin phosphate and a riboflavin-adenine-dinucleotide. A great many flavin compounds have been synthesized and tested for vitamin activity. The substitution of other monosaccharides for *d*-ribose, in general, yielded inactive compounds. Elimination of a methyl group or substitution of one methyl by an ethyl group resulted in reduced activity, while absence of both methyl groups was accompanied by high toxicity (351).

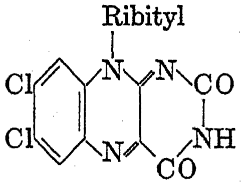
On the basis of mixed-crystal formation between methyl- and chloro-substituted compounds in the benzene series, Kuhn, Weygand, and Möller (209) prepared 6,7-dichloro-9-(*d*-1′-ribityl)isoalloxazine (dichloroflavin). Although this analogue possessed no vitamin activity, it was found to be a potent riboflavin antagonist. With the exception of yeast, the growth of all the organisms tested was inhibited by approximately 0.01 mg./cc. The antibacterial action was observed both with organisms requiring preformed riboflavin and with bacteria not dependent on an external source of the vitamin. A number of other analogues were tested for antiriboflavin action, but none of these possessed more than slight activity. Kuhn *et al.* observed that the reversing action of riboflavin on dichloroflavin varied with time. The following ratios were obtained for 50 per cent of normal growth:

TABLE 14

Bacteriostatic activity and molecular inhibition ratios of triazolopyrimidines (341)

	COMPOUND	ANALOGUE OF	M.E.C.		INHIBITION*	
			E. coli	*Staph. aureus*	By	Ratio
			mg./100 cc.	*mg./100 cc.*		
I	7-Amino-1-*v*-triazolo-[*d*]-pyrimidine	Adenine	8	128	A H	640 640
II	5-Amino-7-hydroxy-1-*v*-triazolo[*d*]pyrimidine	Guanine	64	4	G X	128 35
III	7-Hydroxy-1-*v*-triazolo-[*d*]-pyrimidine	Hypoxanthine	64	16	A H	6400 6400
IV	5,7-Dihydroxy-1-*v*-triazolo-[*d*]pyrimidine	Xanthine	128	128		

* With *E. coli*: A, adenine; H, hypoxanthine; G, guanine; X, xanthine. Ratio indicates number of moles of antagonist required to prevent visible growth in the presence of one mole of purine.



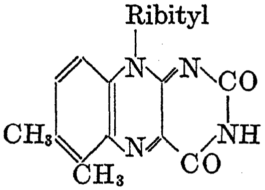
6,7-Dichloro-9-(*d*-1′-ribityl)isoalloxazine

TIME	RATIO DICHLOROFLAVIN:RIBOFLAVIN	
	Staphylococcus aureus	*Streptobacterium plantarum*
days		
2		25:1
3	50:1	60:1
4	70:1	130:1
6	280:1	165:1

When the redox potentials of the vitamin and antivitamin were compared, it was found that the dihydro derivative of dichloroflavin was a considerably weaker reducing agent. At pH 7 the values were:

6,7-Dichloro-9-(*d*-1′-ribityl)isoalloxazine $E_0 = 0.095$ volt
6,7-Dimethyl-9-(*d*-1′-ribityl)isoalloxazine $E_0 = 0.185$ volt

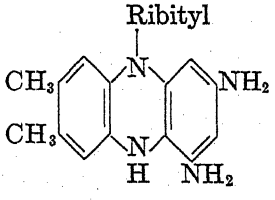
Emerson and Tishler (105) reported that isoriboflavin (5,6-dimethyl-9-(*d*-1′-ribityl)isoalloxazine) suppressed growth both in riboflavin-deficient rats and in animals receiving a suboptimal intake of the vitamin. The growth-depressing effect of 2 mg. per day of isoriboflavin was almost completely overcome by the daily administration of 40 γ of riboflavin.



Isoriboflavin

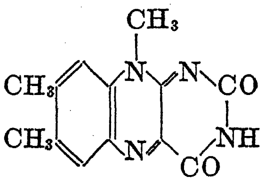
With *L. casei* Foster (129) was unable to demonstrate any growth inhibition with isoriboflavin, even when it was tested at 100,000 times the concentration of riboflavin. In growth experiments with the same organism the isomer showed negligible (less than 0.5 per cent) riboflavin activity. Similar results were obtained by Sarett (359), who found that 1-ribitylamino-2-amino-4,5-dimethylbenzene also possessed only very slight riboflavin activity for the same organism.

Woolley (448) prepared 2,4-diamino-7,8-dimethyl-10-*d*-ribityl-5,10-dihydrophenazine, which is also structurally related to riboflavin. This compound inhibited the growth of *L. casei*, while the corresponding dinitro derivative did not. The growth inhibition, measured by determining the pH of the culture medium,



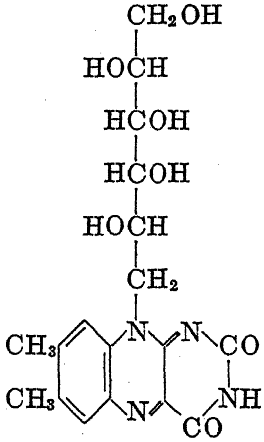
2,4-Diamino-7,8-dimethyl-10-*d*-ribityl-5,10-dihydrophenazine was prevented by appropriate amounts of riboflavin. Because of the instability of the diamino compound, the dinitro derivative was frequently reduced *in situ* with finely divided iron. With one exception, Woolley found that organisms not requiring preformed riboflavin for growth were not inhibited by the diaminophenazine. Signs of a riboflavin deficiency (reduced rate of growth, unkempt fur, and sometimes hyperirritability) were produced by both the dinitro and the diamino derivatives. Sufficient amounts of riboflavin prevented the appearance of these deficiency signs.

Sarett (359) reported that lumiflavin had both an inhibitory and a stimulatory action on the use of riboflavin for growth by *L. casei*. In the presence of minimal amounts of riboflavin, lumiflavin (10–300 γ/10 cc.) inhibited growth. As the concentration of riboflavin was increased, however, a zone was reached where lumiflavin exerted no effect, and finally with still more metabolite lumiflavin showed an augmentative action on growth.



Lumiflavin

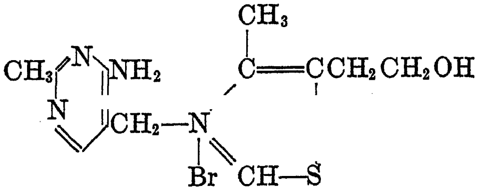
The effect of galactoflavin (6,7-dimethyl-9-(*d*-1′-dulcityl)isoalloxazine) on the growth and survival of young rats was investigated by Emerson, Wurtz, and Johnson (105a). The antagonist was found to suppress the growth and increase the mortality rate of rats on a riboflavin-free diet. Furthermore, the growth of animals receiving a normal supplement of the vitamin (40 γ) was markedly suppressed by the simultaneous administration of 2.16 mg. of galactoflavin. Larger quantities of riboflavin almost completely counteracted this effect.



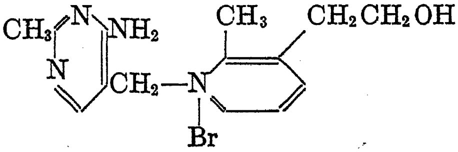
Galactoflavin

J. Thiamine; cocarboxylase

Like nicotinamide and riboflavin, thiamine is an integral part of a coenzyme of vital importance in cell metabolism (400). Cocarboxylase (the pyrophosphoric acid ester of thiamine), together with its apoenzyme[13], catalyzes the oxidation of pyruvic acid to acetic acid and carbon dioxide. A number of microörganisms require preformed thiamine and, of course, it is well known as a vitamin for higher animals. Although many substances chemically related to thiamine have been tested for vitamin activity, only a few have been investigated as antagonists.


Thiamine

Robbins (339) studied the effect on fungi of a thiamine isoster, 2-methyl-4-amino-5-pyrimidylmethyl(2-methyl-3-β-hydroxyethyl)pyridinium bromide, for which Woolley and White (457) proposed the name pyrithiamine. This compound was first synthesized by Tracy and Elderfield (413). Three species of fungi with varying synthetic abilities were employed by Robbins (339). The growth of *Phytophthora cinnamomi*, which required preformed thiamine, was inhibited by pyrithiamine. Thiamine prevented the growth inhibition but the pyrimidine and thiazole portions of the thiamine molecule were ineffective in this respect. *Phthiomorpha gonapodioides*, unable to synthesize the pyrimidine portion of thiamine, grew well in the presence of small concentrations of pyrithiamine, but higher concentrations were inhibitory. Either thiamine or its pyrimidine moiety reversed the growth inhibition, the former being the more effective. With *Phycomyces Blakesleeanus* (able to combine the pyrimidine and thiazole portions but unable to synthesize either), no improvement in growth was observed with small concentrations of pyrithiamine and an inhibitory effect was obtained with larger amounts. Small quantities of pyrithiamine, which did not stimulate growth, produced good growth when combined with the thiazole portion. Robbins concluded that both of the latter fungi might obtain the pyrimidine moiety of thiamine by splitting pyrithiamine.


Pyrithiamine

---

[13] The combination of coenzyme and apoenzyme (specific protein) is frequently referred to as the holoenzyme. In this case the holoenzyme is called carboxylase.

The relationship between thiamine and pyrithiamine was investigated further by Woolley and White (457, 458). In mice, they observed that pyrithiamine produced characteristic signs of thiamine deficiency. As little as 20 γ of pyrithiamine per day eventually produced deficiency symptoms and the death of the animals. All of the symptoms could be prevented or cured with appropriate amounts of thiamine.

Woolley and White (458) also studied the effect of pyrithiamine on the growth of a number of species of bacteria, yeasts, and fungi. These investigators observed that organisms requiring preformed thiamine for maximal growth were unable to grow in the presence of small amounts of pyrithiamine. Thiamine prevented this growth inhibition, one part of the vitamin reversing ten parts of pyrithiamine. Strains which required only the pyrimidine or thiazole portions of the thiamine molecule were less susceptible. In these cases the inhibition ratio was about 1000:1. With organisms such as *E. coli*, for which thiamine was not a growth stimulant, pyrithiamine appeared to be ineffective even in concentrations 500,000 times as great as the amounts which inhibited the growth of sensitive organisms (table 15). The resistance of insusceptible bacteria could not be explained on the basis of the amount of thiamine synthesized or the production of some other reversing agent by the resistant organisms.

TABLE 15

Inhibition ratio of pyrithiamine for various microörganisms (458)

ORGANISM	INHIBITION* RATIO	THIAMINE REQUIREMENT
Ceratostomella fimbriata	7	Intact thiamine
Ceratostomella pennicillata	10	Intact thiamine
Phytophthora cinnamomi	12	Intact thiamine
Endomyces vernalis	130	Pyrimidine†
Mucor ramannianus	800	Thiazole‡
Saccharomyces cerevisiae	800	Pyrimidine and thiazole
Staphylococcus aureus	2000	Pyrimidine and thiazole
Salmonella gallinarum	1000	Pyrimidine and thiazole
Escherichia coli	2,000,000	None
Lactobacillus casei	5,000,000	None
Streptococcus fecalis R	5,000,000	None

* Half-maximum inhibition.

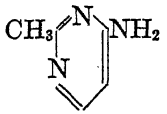
† 2-Methyl-4-amino-5-ethoxymethylpyrimidine.

‡ 4-Methyl-5-hydroxyethylthiazole.

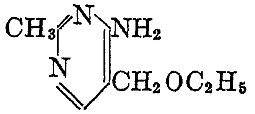
In a similar investigation with *Staph. aureus*, Wyss (462) obtained an inhibition ratio of pyrithiamine to thiamine of approximately 700:1 for half-maximum growth. He also found that his strain of *E. coli* was susceptible to pyrithiamine, although in this case the ratio was about 20,000:1. Wyss reported that noninjurious amounts of pyrithiamine failed to produce antibacterial concentrations in the blood of mice.

With a pyrithiamine-fast strain of yeast, Woolley (449) demonstrated that the resistance could be explained, at least in part, by a breakdown of pyrithiamine by the yeast cells to yield the pyrimidine portion (2-methyl-4-amino-5-hydroxymethylpyrimidine) of thiamine. Both the parent yeast and the resistant strain could utilize the pyrimidine portion as well as intact thiamine. Since the fast strain grew more slowly in the absence of pyrithiamine, Woolley suggested that indirectly this antagonist served as a growth factor for the resistant yeast. His strain of *E. coli* also degraded the pyrithiamine molecule, an observation which might explain the variation in the susceptibility of different strains of this organism.

Sarett and Cheldelin (360) found that less pyrithiamine was required to inhibit the growth of *Lactobacillus fermentum* and *Penicillium digitatum* when cocarboxylase was used as a growth stimulant than when thiamine was employed. Large quantities of 2-methyl-4-aminopyrimidine and 2-methyl-4-amino-5-ethoxymethylpyrimidine also inhibited growth, and again less of either compound was required when cocarboxylase was the growth stimulant. The authors suggested that these results indicate that thiamine is attached to its apoenzyme before being phosphorylated, and that cocarboxylase formed from thiamine is more firmly bound than that formed from added diphosphothiamine.

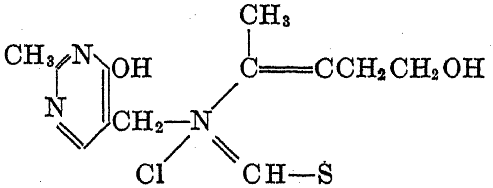


2-Methyl-4-aminopyridine



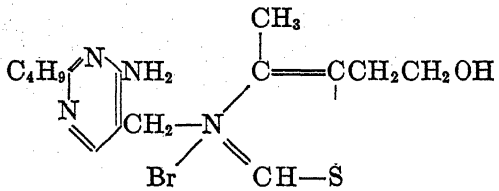
2-Methyl-4-amino-5-ethoxymethyl-pyrimidine

Oxythiamine (33), in which the amino group is replaced by hydroxyl, has been reported by Soodak and Cerecedo (388) to be toxic to mice on a low-thiamine diet. It was also found to inhibit the fish principle (enzyme) which causes Chastek paralysis by destruction of thiamine.



Oxythiamine

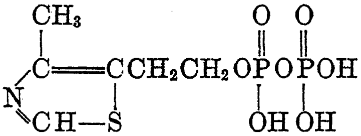
The *n*-butyl homologue of thiamine, 2-*n*-butyl-4-amino-5-pyrimidylmethyl-(2-methyl-3-β-hydroxyethyl)thiazolium bromide, was shown to possess antithiamine activity for rats by Emerson and Southwick (104a). Deficiency symp-



"Butylthiamine"

toms such as polyneuritis developed in the animals receiving a diet containing a suboptimal level of the vitamin and 2.8 mg. of "butylthiamine". Control rats on the same diet all survived and were free from polyneuritis. Additional thiamine counteracted the effect of the butyl homologue in a ratio of approximately 40:1. The authors called attention to previous results indicating that the ethyl and *n*-propyl homologues, particularly the former, possess thiamine activity.

Buchmann, Heegaard, and Bonner (52) observed that the thiazole pyrophosphate portion of the cocarboxylase molecule markedly inhibited the enzymatic decarboxylation of pyruvic acid by dried brewer's yeast. The thiazole moiety alone, its monophosphate ester, or sodium pyrophosphate produced no inhibition. In the presence of 4 γ of cocarboxylase, 80 γ of "thiazole pyrophosphate" reduced the amount of carbon dioxide produced to 10 per cent of the control value. Buchmann *et al.* suggested that this inhibition was due to a competition between cocarboxylase and "thiazole pyrophosphate" for the specific carboxylase protein. To support this interpretation, they showed that if the "thiazole pyrophosphate" was added prior to cocarboxylase, the rate of carbon dioxide production was lower initially than when the coenzyme and antagonist were added simultaneously.

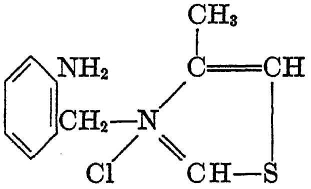


"Thiazole pyrophosphate"

Sevag, Shelburne, and Mudd (372) reported that sulfathiazole inhibited the carboxylase system of *Staph. aureus* and *E. coli* to a greater extent than several other sulfonamides, including sulfanilamide, sulfapyridine, and sulfadiazine. At 0.0055 *M* sulfathiazole produced a 50 per cent inhibition, while with the other sulfonamides the inhibition was less than 10 per cent. The authors considered that the presence of the thiazole ring in sulfathiazole might account for the greater inhibition produced by this compound. In some cases the results appeared to correlate with the relative bacteriostatic action of the sulfonamides, although sulfamerazine had no effect on the carboxylase system.

In later studies, Sevag and coworkers (369, 370, 373) found that the partial inhibition of yeast or *Staph. aureus* carboxylase by sulfathiazole could be partly overcome with cocarboxylase. With dried brewer's yeast, for example, 0.0055 *M* sulfathiazole produced a 25 per cent inhibition which was 44 per cent reversed by 6.8×10^{-7} *M* cocarboxylase (373). *p*-Aminobenzoic acid (PABA) also inhibited the carboxylase system of *Staph. aureus* and of *E. coli.* Even so, under some conditions PABA prevented the inhibition of the carboxylase of the same organisms by sulfathiazole (369). Neopeptone counteracted the inhibitory effect of sulfanilamide on *E. coli* carboxylase, but showed no effect on PABA inhibition (370). Sevag and associates considered these results to provide additional evidence for the hypothesis that the mode of action of sulfanilamide-type compounds could be explained most readily on the basis of their effect on the respiratory enzymes. They pointed out that in their experiments PABA behaved like an inhibitor of catalysis and not like a catalyst.

During a study of the enzymatic nature of the fish principle which destroys thiamine, Sealock and Goodland (365) found that 3-(*o*-aminobenzyl)-4-methylthiazolium chloride (70) inhibited the breakdown of thiamine *in vitro*. Several other thiazolium chloride derivatives less closely related structurally to thiamine were found to be only partly inhibitory or inactive, whereas I in 0.0005 *M* concentration completely prevented the destruction of an equal amount of thiamine[14]. Sealock and Goodland demonstrated that the extent of the inhibition of thiamine destruction was dependent on the ratio of the vitamin to the inhibitor. By use of the Lineweaver–Burk method, they showed that the benzylthiazolium compounds specifically inhibited thiamine destruction by competing with the vitamin for the enzyme. Calculation of the Michaelis constants indicated that the inhibitor compound combined somewhat more firmly with the fish principle than did thiamine.

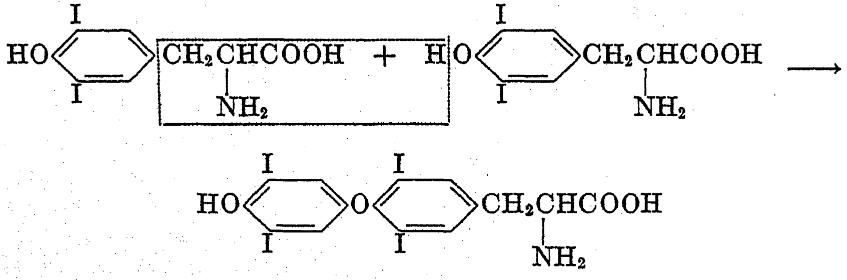


I

3-(*o*-Aminobenzyl)-4-methylthiazolium chloride

K. Thyroxine

The active principle of the thyroid gland controls the metabolic rate of higher animals, and probably affects all tissue cells. Under the stimulus of the thyrotropic hormone of the anterior pituitary, thyroid hormone may be formed directly by the iodination of a polypeptide. Another possibility is the production of thyroxine through the iodination of tyrosine followed by a combination of two molecules of 3,5-diiodotyrosine as indicated (157, 158):



Thyroxine

---

[14] In this case, of course, the benzylthiazolium derivative (I) cannot be regarded as an antagonist of thiamine function, although future studies may show it to have such an action.

Overactivity of the thyroid gland in man results in hyperthyroidism, and much of the interest in thyroxine antagonists has been due to their application to the treatment of this condition (14, 174, 435). Numerous substances, mostly of biological origin, have been reported to inactivate the thyroid hormone or to suppress its formation in the thyroid gland (322). However, with the exception of those which are neutralized by iodine, no clear evidence regarding their action in relation to thyroxine has been advanced. Consequently, only substances of known structure which appear to inhibit the synthesis of thyroxine are covered in this review.

During a nutritional study with sulfaguanidine, Mackenzie, Mackenzie, and McCollum (271) observed a marked enlargement of the thyroid glands of rats receiving 1 or 2 per cent of sulfaguanidine in a purified diet. Striking histological changes in the glands were also found. The effects on the thyroid were not prevented by *p*-aminobenzoic acid or yeast. Richter and Clisby (337) noted a similar hyperplasia of the thyroid in rats fed phenylthiourea. They suggested that the hyperactive picture of the thyroid gland might result from the efforts of the animals to compensate for the reduction of metabolism produced by this substance. An attempt to isolate the goitrogenic substance present in rape seed led Kennedy (194) to study allylthiourea. He found that small (20 mg.) daily doses of this compound for eight weeks caused a three- to four-fold increase in the weight of the thyroid gland of rats.

Following up their earlier observations, Mackenzie and Mackenzie (269, 270) reported that, in addition to sulfaguanidine, a number of other sulfonamides as well as thiourea and several of its derivatives produced an enlargement of the thyroid glands of rats, mice, and dogs. The basal metabolic rate of rats receiving sulfaguanidine was reduced to −20 per cent. Sodium iodide was ineffective in preventing these effects, but small amounts of thyroxine or desiccated thyroid entirely prevented the thyroid enlargement and histologic changes. After removal of the thyroid gland, sulfaguanidine did not further depress the basal metabolic rates nor inhibit the response to small doses of thyroxine. Hypophysectomy likewise eliminated the thyroid alteration produced by the drug. On the basis of this evidence, the Mackenzies (270) concluded that the sulfonamides and thioureas probably exerted a depressing influence primarily on the functional activity (thyroxine formation) of the thyroid, and that thyroid enlargement was a reflection of increased pituitary activity resulting from this depression. Table 16 summarizes some of the results obtained with active compounds.

A number of other substances, most of which were inactive, were also investigated for antithyroid action (270). N^4-Benzylsulfanilamide, in which the aromatic amino group is substituted, was practically inactive. Similarly, desaminosulfamethyldiazine was inert, although the parent compound with the amino group present showed activity. *p*-Aminobenzoic acid also showed a slight effect. In contrast to thiourea and its diethyl and allyl derivatives, acetylthiourea and thioacetamide were reported to be inactive, as were urea and phenylurea. Negative results were also obtained with cystine, cysteine, guanidine carbonate, sulfanilic acid, sulfonal, thiamine, and sodium and ammonium thiocyanates.

Astwood, Sullivan, Bissell, and Tyslowitz (19), after a very similar independent study, reached the same conclusions as Mackenzie and Mackenzie (270) regarding the mode of action of several sulfonamides and thiourea. They listed the compounds investigated in the following order of decreasing antithyroid activity: thiourea, sulfadiazine, sulfapyridine, sulfathiazole, sulfaguanidine, sulfanilylurea, sulfanilamide, and N^4-succinylsulfathiazole. Not only was the goitrogenic action of sulfaguanidine completely prevented by desiccated thyroid powder, but the drug also had no influence on the toxic or calorigenic action of thyroid hormone in normal or hypophysectomized rats. Astwood and coworkers concluded that the compounds under investigation acted by interfering with the synthesis of thyroid hormone. The sequence of events was considered to be as follows:

TABLE 16

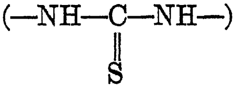
Effect of various compounds on the thyroid of young rats (270)

COMPOUND	PER CENT IN DIET	DAYS ON DIET	THYROID WEIGHT
			mg./100 g.
Sulfanilamide	1	58	14.0
Sulfathiazole	1.5	14	12.5
Sulfapyridine	1	14	12.0
Sulfapyridine	1	21	29.0
Sulfadiazine	0.75	45	47.0
Sulfamethyldiazine	0.75	45	25.0
Thiourea	0.05	14	13.0
Thiourea	0.5	14	37.4
Diethylthiourea	1	28	26.4
Allylthiourea	0.5	14	16.4
Controls		50	8*

* Approximate value.

> "Shortly after the drug is administered, the organism becomes unable to synthesize thyroid hormone at a normal rate, and the quantity of circulating hormone tends to fall. In response to this deficit an excess of thyrotropin is produced by the pituitary which stimulates the thyroid to hyperplasia and to the release of the normal thyroid hormone stored therein. Within 48 hours of the first administration of the drug these compensatory changes are histologically visible, and for a number of days this mechanism is adequate to maintain the metabolic rate at a normal level. Eventually, however, the store of normal thyroid hormone is exhausted, as evidenced by a complete loss of demonstrable colloid at the end of 7 to 10 days, and as new hormone can be made only at a reduced rate, the metabolic rate falls even though the thyroid hyperplasia is still advancing. The variable period observed to be required before the metabolic rate falls may be related to environmental or other factors which alter the thyroid hormone requirement and thus the time

necessary to deplete the normal stores. The actual level of hypometabolism reached under the influence of the drug may depend upon the degree to which the process of thyroid hormone synthesis is hindered, and may depend upon the dose and upon the efficiency of the compound in question."

A large number of compounds were investigated for antithyroid activity by Astwood (15), who found that the active substances fell into two general classes, compounds with the thioureylene grouping  $(\text{—NH—}\underset{\underset{\displaystyle \mathrm{S}}{\|}}{\mathrm{C}}\text{—NH—})$ and certain derivatives of aniline. The most potent compounds belonged to the former class. Of these, 2-thiouracil and 5,5-diethyl-2-thiobarbituric acid[15] were the most active. Some of the data on a selected group of the thiourea derivatives tested are recorded in table 17. The antithyroid activity was graded as + to ++++ on the basis of the microscopic appearance of the thyroid glands after administration of the substances for 10 days in the food or drinking water.

In general, alkyl and aryl derivatives were not more active than thiourea itself, and most of them were more toxic, although *sym*-diethylthiourea was an exception. Substituted 2-thiohydantoins were more potent than thiourea but also considerably more toxic. 2-Aminothiazole also possessed some activity. Several other 5-substituted barbituric acids tested were less effective than the 5,5-diethyl derivative. In addition to the sulfonamides, a group of other aniline derivatives was studied. The *o*, *m*, and *p*-aminobenzoic acids, *p*-aminoacetanilide, and *p*-aminophenylacetic acid showed varying degrees of effectiveness, but with the exception of the sulfonamides none of the other aniline derivatives tested was active. Inorganic thiocyanates produced enlargement of the thyroid glands on prolonged administration; however, this effect was completely prevented by iodide. A large group of inactive compounds related to the active types was also reported by Astwood (15). These included uracil and several other pyrimidines, benzoic and *p*-nitrobenzoic acids, tyrosine, benzene and *p*-toluenesulfonamide, 3,5-diiodo-4-hydroxybenzoic acid, and a number of organic sulfur compounds. As a working hypothesis it was suggested that the active aniline derivatives acted through a competitive mechanism with the enzyme system responsible for the conversion of diiodotyrosine to thyroxine. The thiourea derivatives were considered as possible specific inhibitors of the same system.

Chapman (62) compared the antithyroid activity of 6-methyl-2-thiouracil with that of the parent compound and reported that the former was at least as active as thiouracil. Higgins (172) investigated the action of 2-sulfanilyl-5-aminothiazole (promizole), which he found to resemble thiourea and some of the sulfanilamides in its effect on thyroid activity. The decrease in metabolic rate

---

[15] This compound was originally reported as 2-thiobarbituric acid but a subsequent investigation by Astwood, Bissell, and Hughes (Endocrinology **36**, 72 (1945)) indicated an error in labeling, and 2-thiobarbituric acid was found to be inactive.

TABLE 17

Antithyroid activity of derivatives of thiourea (15)

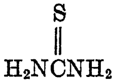
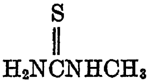
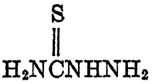
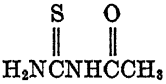
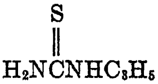
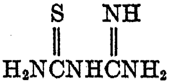
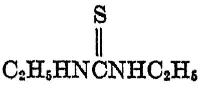
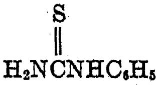
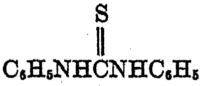
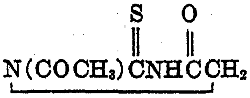
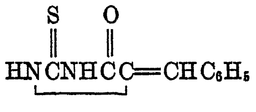
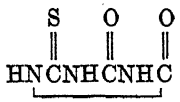
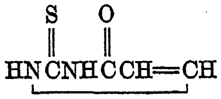
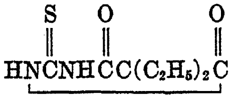
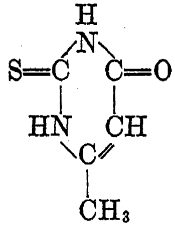
COMPOUND	FORMULA	AVERAGE DOSE*	ACTIVITY
Thiourea	 $H_2N\overset{\overset{S}{\|}}{C}NH_2$	2.1	+
		13.1	++++
Methylthiourea	 $H_2N\overset{\overset{S}{\|}}{C}NHCH_3$	4.6	++
		44.4	++++
Thiosemicarbazide	 $H_2N\overset{\overset{S}{\|}}{C}NHNH_2$	5.1	+
Acetylthiourea	 $H_2N\overset{\overset{S}{\|}}{C}NH\overset{\overset{O}{\|}}{C}CH_3$	4.0	+
		13.0	+++
Allylthiourea	 $H_2N\overset{\overset{S}{\|}}{C}NHC_3H_5$	2.1	+
		4.9	+++
Guanylthiourea	 $H_2N\overset{\overset{S}{\|}}{C}NH\overset{\overset{NH}{\|}}{C}NH_2$	5.1	−
sym-Diethylthiourea	 $C_2H_5HN\overset{\overset{S}{\|}}{C}NHC_2H_5$	0.6	+
		2.5	+++
Phenylthiourea	 $H_2N\overset{\overset{S}{\|}}{C}NHC_6H_5$	2.5	−
sym-Diphenylthiourea	 $C_6H_5NH\overset{\overset{S}{\|}}{C}NHC_6H_5$	530	++
Methylisothiourea sulfate	$(HN{=}C(SCH_3)NH_2)_2 \cdot H_2SO_4$	11.1	++
1-Acetyl-2-thiohydantoin	 $\underbrace{N(COCH_3)\overset{\overset{S}{\|}}{C}NH\overset{\overset{O}{\|}}{C}CH_2}$	3.0	+
		9.7	+++

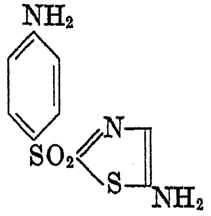
TABLE 17—*Continued*

COMPOUND	FORMULA	AVERAGE DOSE*	ACTIVITY
5-Benzal-2-thiohydantoin........	 $\mathrm{HN\overset{\overset{S}{\|}}{C}NH\overset{\overset{O}{\|}}{C}C{=}CHC_6H_5}$	1.3 16.5	+ ++++
2-Thiocyanuric acid............	 $\mathrm{HN\overset{\overset{S}{\|}}{C}NH\overset{\overset{O}{\|}}{C}NH\overset{\overset{O}{\|}}{C}}$	30.0	−
2-Thiouracil....................	 $\mathrm{HN\overset{\overset{S}{\|}}{C}NH\overset{\overset{O}{\|}}{C}CH{=}CH}$	0.6 2.3 3.6	+ ++ ++++
5,5-Diethyl-2-thiobarbituric acid.	 $\mathrm{HN\overset{\overset{S}{\|}}{C}NH\overset{\overset{O}{\|}}{C}C(C_2H_5)_2\overset{\overset{O}{\|}}{C}}$	0.6 3.6 17.2	+ ++ ++++

*Milligrams per 100 g. of body weight per day in food or drinking water.



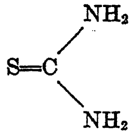
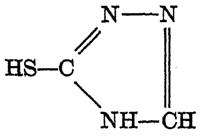
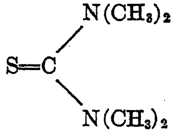
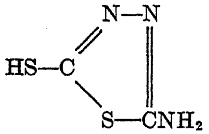
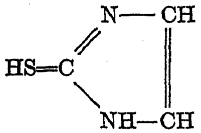
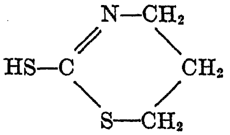
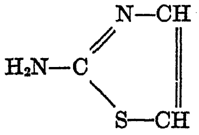
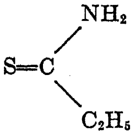
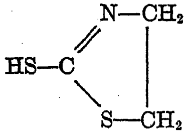
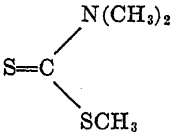
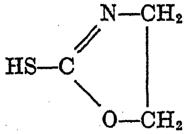
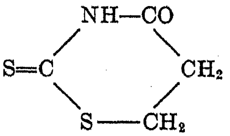
6-Methyl-2-thiouracil



2-Sulfanilyl-5-aminothiazole

and other changes induced by promizole could be prevented by thyroxine and desiccated thyroid but not by sodium iodide.

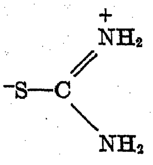
In a subsequent study, Astwood, Bissell, and Hughes (16, 18) examined an additional group of over two hundred compounds. An improved method of assay (17), based on the weight and total iodine content of the thyroid glands of rats, was employed. Although not entirely satisfactory, this method permitted a somewhat more accurate comparison of the compounds. Thiouracil was assigned an arbitrary antithyroid activity of 1.0, and the relative activities of a representative group of organic thio compounds were listed as follows (16):

COMPOUND	RELATIVE ACTIVITY (THIOURACIL = 1.0)	COMPOUND	RELATIVE ACTIVITY (THIOURACIL — 1.0)
 Thiourea	0.1	 3-Mercapto-1,2,4-triazole	0.3
 Tetramethylthiourea	0.3	 2-Mercapto-5-amino-1,3,4-thiadiazole	1.6
 2-Mercaptoimidazole	1.5	 2-Mercapto-5,6-dihydro-1,3,4-thiazine	0.1
 2-Aminothiazole	0.1	 Thiopropionamide	<0.01
 2-Mercaptothiazoline	1.3	 Methyl N,N-dimethyldithiocarbamate	<0.01
 2-Mercaptoöxazoline	0.2	 Propiorhodanine	<0.01

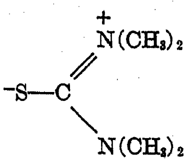
Substitution of all four hydrogens in thiourea increased activity, although a tautomeric equilibrium between the thio and mercapto forms of this compound is impossible[16]. Isothioureas were ineffective, but incorporation of the sulfur

---

[16] Presumably tetramethylthiourea can still exist in resonance forms similar to thiourea: e.g.,



and

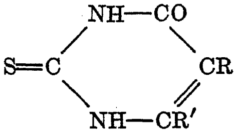


atom in a heterocyclic ring (e.g., 2-aminothiazole) did not abolish activity. The large number of inactive thio compounds investigated suggested that this grouping alone could not account for antithyroid activity. In the 2-thiobarbituric acid series it was found that active compounds were obtained only when both hydrogens in the 5-position were replaced. The dimethyl derivative was about one-tenth as active as thiouracil, while 5,5-diethyl-2-thiobarbituric acid was the most potent member of this series (antithyroid activity = 1.7).

Of all the compounds tested by Astwood *et al.* (18), the most effective ones were found among the alkyl derivatives of thiouracil synthesized by Anderson, Halverstadt, Miller, and Roblin (11). In this series, also, a great variation in activity with changes in structure was observed, the introduction of highly polar groups reducing the antithyroid action practically to zero. Some of the results obtained with this group of compounds are recorded in table 18.

TABLE 18

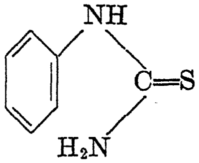
The relative antithyroid activity of various substituted thiouracils (18)



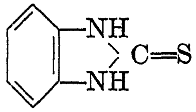
R	R′	ACTIVITY
—H	—H	1.0
—C_2H_5	—H	3.5
—$C_3H_7(n)$	—H	2.0
—$C_4H_9(n)$	—H	0.6
—H	—C_2H_5	8.0
—H	—$C_3H_7(n)$	11.0
—H	—$C_4H_9(n)$	3.0
—H	—$CH_2C_6H_5$	10.0
—H	—C_6H_5	1.0
—C_2H_5	—C_2H_5	2.0
—CN	—H	<0.001
—$COOC_2H_5$	—H	<0.001
—H	—CF_3	<0.01
—H	—NH_2	<0.001

A group of 56 compounds was tested for antithyroid activity by McGinty and Bywater (248). A number of these were also tested by Astwood *et al.* (18), and in general the results of the two studies were in good agreement. Thiourea and substituted thioureas were less active than thiouracil. Of the substituted thiouracils, the 4-methyl derivative appeared to be slightly more active than the parent compound, while the *N*-phenyl derivative showed only slight activity. Using a somewhat different method of assay and a different base line (thiouracil = 100), McGinty and Bywater found the antithyroid activity of 2-mercaptothiazoline to be 131, 2-mercaptobenzimidazole 116, and 2-mercaptopyridine

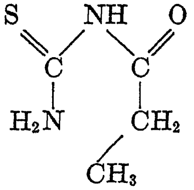
23. They pointed out an interesting analogy between open-chain and ring compounds, as follows:



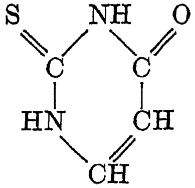
Phenylthiourea (14)



2-Mercaptobenzimidazole (116)



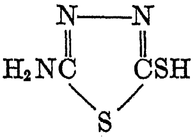
Propionylthiourea (14)



Thiouracil (100)

It was suggested (248) that the higher antithyroid activity (values given in parentheses) of the cyclic compounds in each case might be due to their tendency to exist primarily in the mercapto form.

Bywater, McGinty, and Jenesel (56) prepared and studied a series of 2-mercaptobenzimidazoles and 2-mercaptoimidazolines. None of the derivatives in either group was as active as the parent compound. The substitution of halogens, alkoxyl groups, or alkyl groups reduced the antithyroid action of the former compound. Similarly, replacing the mercapto group by an amino lowered the activity from 116 to 6.



2-Mercapto-5-amino-1,3,4-thiadiazole

McGinty and Bywater (248a) also examined the antithyroid action of a number of sulfones and related compounds. Alkyl and unsubstituted phenyl sulfones were devoid of activity, whereas *p*-aminophenyl sulfones possessed some degree of action. Promizole was the most active of the sulfones, although the corresponding sulfide was slightly more effective. Most active of the compounds studied was the unsubstituted mercapto compound, 2-mercapto-5-amino-1,3,4-thiadiazole, with an antithyroid activity of 156.

Several series of thio compounds were investigated by Williams and Frame (435a). These investigators reported that no goitrogenic effect was exerted by a number of miscellaneous non-thiourea sulfur compounds, isothioureas, and thioureas in which the sulfur atom was incorporated in ring structures. *N*-Substitution of thiourea also reduced or abolished activity in most cases. The most active compounds studied were listed in the following tentative order of decreasing effectiveness: 6-methylthioureacil, thiouracil, *o*-phenylenethiourea, 5-methylthiouracil, ?-benzylthiouracil, 6-phenylthiouracil, tetramethylthiourea, diethylthiourea, and thiourea.

Numerous studies attempting to define more exactly the mechanism of action of these types of antithyroid compounds have been made. In reviewing this aspect of the work, Astwood (16) indicated that, beyond an interference with the synthesis of thyroxine, a more precise explanation involved considerable speculation. The lack of understanding of the natural mechanism of thyroxine formation has been a limiting factor in these studies. The oxidation of iodide to iodine, which is presumably a prerequisite to the formation of organic iodine, requires an oxidizing system of relatively high potential, such as a peroxidase and hydrogen peroxide (16). Dempsey (81) presented histochemical evidence indicating that fine granules in the thyroid cells contain a peroxidase which is inhibited by dilute solutions of thiouracil. However, Glock (143) was unable to isolate a peroxidase from thyroid tissue by chemical methods. Employing redox indicators, De Robertis and Gonçalves (83) reported that the oxidation–reduction potential of the normal thyroid cell was above $+0.050$ volt, while that of the colloid was about -0.20 volt. In activated cells the colloid potential rose to the same level as that of the cells ($+0.050$ volt). Thiourea ($M/200$) lowered the potential of both the cells and the colloid of the activated gland to -0.20 volt. On the other hand, sulfanilamide did not affect the normal oxidation–reduction potential of the thyroid cells.

Franklin and Chaikoff (131, 132) investigated the effects of various sulfonamides on the *in vitro* conversion of inorganic iodide to diiodotyrosine and thyroxine by surviving thyroid tissue slices with the aid of radioactive iodide. They found that the sulfonamides ($10^{-3}M$) inhibited the conversion, and that the degree of inhibition was related to the concentration of sulfonamide. On the other hand, sulfanilamide did not appreciably depress the iodine-concentrating capacity of surviving thyroid slices. Similar *in vitro* results using radioactive iodide were obtained with thiourea and thiouracil (133), although several groups of investigators (77, 134, 195, 334) reported that these antithyroid compounds markedly inhibit the accumulation of radioactive iodide by the thyroid gland *in vivo*. Miller, Anderson, Madison, and Salley (294) have sounded a word of warning concerning the interpretation of studies with radioactive iodide. These investigators observed a rapid exchange, under certain conditions, between radioactive iodide or iodine and diiodotyrosine.

Both thiourea and thiouracil have been reported to decrease greatly the iodine content of the thyroid (17, 30), but McGinty (247) found that the thyroid glands of animals receiving thiouracil were still capable of concentrating some iodine from large doses of potassium iodide. In the presence of the drug, however, the accumulated iodine was water soluble and did not appear to be converted to thyroid hormone.

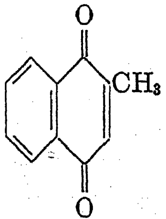
Campbell, Landgrebe, and Morgan (57) suggested that the antithyroid activity of thiourea might be due to its well-known reactivity toward iodine. On this basis, the iodine formed in the thyroid would react so rapidly with thiourea that it would not be available for conversion to diiodotyrosine and thyroxine. The same concept was examined experimentally by Miller, Roblin, and Astwood (295) with respect to thiouracil and other thio compounds. It was found that in neutral buffered solutions thiouracil reacted almost instantaneously with one equivalent of iodine to form a disulfide which could be isolated in almost quantitative yields as the disodium salt. Tyrosine and casein could not be iodinated in the presence of thiouracil, but sulfonamides reacted with iodine more slowly than tyrosine and the action of these compounds could not be accounted for by this mechanism.

L. Vitamin K; prothrombin

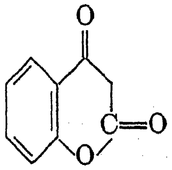
A number of 1,4-naphthoquinones, the simplest of which is the 2-methyl derivative, restore normal prothrombin levels in animals on a vitamin K-deficient diet. Since the prothrombin concentration (which affects blood coagulation) is reduced in this deficiency, it is generally assumed that the K vitamins are involved in the formation of prothrombin (351).

When Stahmann, Huebner, and Link (392) found that 3,3′-methylenebis-(4-hydroxycoumarin), dicumarol, was the hemorrhagic agent in spoiled sweet clover hay which increased prothrombin time, the action of this anticoagulant was investigated from the standpoint of vitamin K antagonism. Link (234) and Quick (329) have reviewed the present evidence on this subject. While the results are not unequivocal, the effect of small single doses of dicumarol appears to be prevented by 2-methyl-1,4-naphthoquinone (50, 79, 228, 314, 374), or vitamin K_1 oxide (80, 244). The mechanism of action of dicumarol is not entirely clear at present, and it is quite possible that the majority of known anticoagulants might better be classified primarily as prothrombin antagonists. Available evidence suggests that dicumarol inhibits the synthesis of prothrombin perhaps by interfering with the utilization of the K vitamins, rather than by any direct action on prothrombin (329).

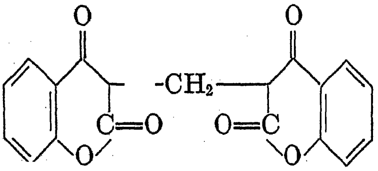
Witts (438) pointed to a basic similarity between the structures of 2-methyl-1,4-naphthoquinone and 4-hydroxycoumarin and suggested that dicumarol might act by interfering with the utilization of vitamin K by the liver.



2-Methyl-1,4-napththoquinone

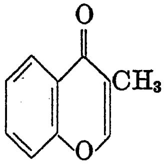


4-Hydroxycoumarin (keto form)



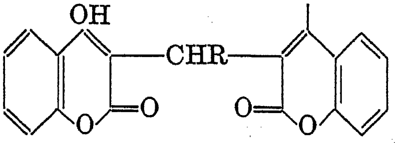
Dicumarol (keto form)

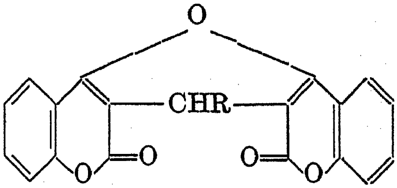
Meunier and Mentzer (289) also called attention to these structural relationships and reported that they were able to prevent completely the effects of dicumarol by repeated daily doses of 2-methyl-1,4-naphthoquinone. They also reported that 4-hydroxycoumarin and 3-methylchromone possessed a slight antihemorrhagic and vitamin K-like action. The methylxanthines—theophylline, theobromine, and caffeine—have been found by Field, Larsen, Spero, and Link (120) to decrease prothrombin time and counteract the hypoprothrombinemic action of dicumarol. However, Quick (329a) was unable to confirm these observations in rabbits and dogs.


3-Methylchromone

In vitamin K-deficient chicks Glavind and Jansen (142) showed that there was a quantitative relationship between the metabolite and dicumarol. The proportions of the two substances could be adjusted so that the rate of prothrombin formation was unaffected by either. With the smallest active doses of the vitamin the ratio was 200–500:1, but the lack of constancy of this ratio precluded the possibility of a simple stoichiometric relationship. It was suggested that dicumarol might exert a specific toxic effect on prothrombin formation in the liver, which was compensated by the stimulating effect of vitamin K.

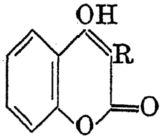
Overman, Stahmann, Huebner, Sullivan, Spero, Doherty, Ikawa, Graf, Roseman, and Link (313) have summarized their extensive studies on the relation of structure to anticoagulant activity among compounds of the 4-hydroxycoumarin type. On the basis of a single oral dose assay, they concluded that 3,3′-methylenebis(4-hydroxycoumarin) was the most potent member of this class of substances. Replacement of a hydrogen of the methylene group by an alkyl or aryl group as indicated in formula I lowered activity more or less in relation to the size of the group.


I

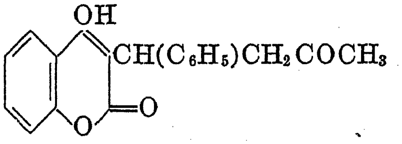

II

Jensen and Jensen (189), Lehmann (227), and Fantl (116) reported the ethylidene derivative (R = CH_3) equal to or less active than dicumarol. Overman *et al.* (313) investigated a large series of compounds of type I. The first two members of the series, the ethylidene and propylidene derivatives, were about one-third as active as dicumarol, while longer chains, phenyl or carboxyl groups, decreased the activity still more. Replacement of the methylene group by a sulfur atom reduced the activity to $\frac{1}{20}$ that of dicumarol. Monoesters and glucosides immediately formed the inactive anhydrides (II) on neutralization. Substitution of the methylene carbon atom facilitated anhydride formation, and might have accounted for the decrease in anticoagulant activity of type I compounds.

Another group of substances studied by Overman and his associates (313) was that of the 3-substituted-4-hydroxycoumarins (III). The methyl derivative of this type has been described as inactive or slightly anticoagulant by several investigators (116, 189, 227, 313), although Meunier and Mentzer (289) reported the compound to have slight vitamin K activity. In general, Overman *et al.* (313) found that compounds of type III, when R was alkyl, possessed very low anticoagulant action. When R was cyano, bromo, nitro, or oximino, the derivatives were inactive. However, the presence of a benzene ring and a ketone in the R group increased the effectiveness, so that 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin (IV) was $\frac{1}{4}$ as active as dicumarol.



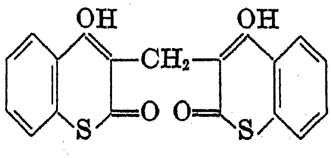
III



IV

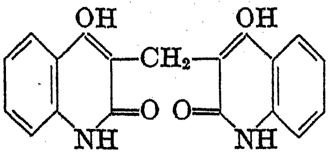
The diesters, ethers, and acetals of 3,3′-methylenebis(4-hydroxycoumarin) investigated by Overman *et al.* (313) showed a lower degree of activity than the parent substance. It was suggested that these compounds owed their action to an *in vivo* breakdown to dicumarol. Substitution of the benzene ring with methyl, bromo, or hydroxyl groups destroyed the activity in practically every case.

Several compounds structurally related to dicumarol were investigated by Mentzer, Meunier, Buu Höi, and Cagniant (288, 290). The sulfur analogue (V) was reported to be $\frac{1}{10}$ as effective, while the corresponding derivative in



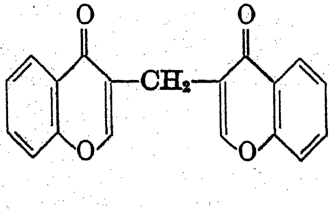
V

3,3′-Methylenebis(4-hydroxy-1-thiocoumarin)

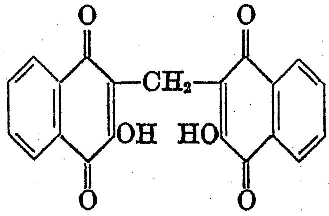


VI

3,3′-Methylenebis(4-hydroxy-2-quinolone)



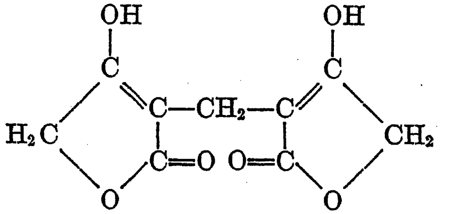
VII

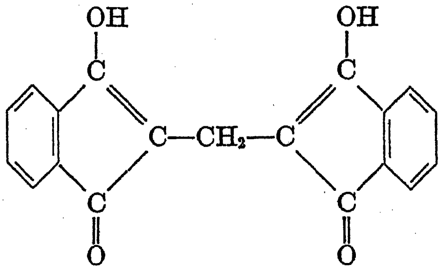


VIII

which the oxygen atoms were replaced by NH's to form a quinolone derivative (VI) was about $_{50}$ as active as dicumarol. In view of the vitamin K activity of 3-methylchromone and phthiocol, these investigators also prepared 3,3′-methylenebischromone (VII) and 3,3′-methylenebis(2-hydroxy-1,4-naphthoquinone) (VIII). The former possessed about $\frac{1}{50}$ the activity of the parent compound; the latter $\frac{1}{25}$, but the action was of short duration.

Jansen and Jensen (189) and Overman *et al.* (313) studied other compounds related to dicumarol, such as methylenebis(tetronic acid) (IX), 3,3′-methylenebis(4-hydroxy-6-methyl-α-pyrone), and 2,2′-methylenebis(indanedione) (X).


IX


X

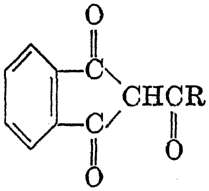
None of these compounds was an anticoagulant. Similarly, 3,3′-methylenebis(coumarin) was inactive, although coumarin itself showed a trace of activity (313). Degradation products of 3,3′-methylenebis(4-hydroxycoumarin) were also without anticoagulant action (189, 313). Overman *et al.* (313) concluded that the minimum structural requirements in this series are an intact 4-hydroxycoumarin residue with the 3-position substituted by a hydrogen atom or a carbon residue. Every compound fulfilling these requirements appeared to have some activity.

When added to blood or plasma *in vitro*, dicumarol does not affect clotting power (58), and its action in experimental animals is detectable only after a lapse of 12 to 24 hr. (315). These facts led Link, Overman, Sullivan, Huebner, and Scheel (235) to suggest that 3,3′-methylenebis(4-hydroxycoumarin) might undergo a chemical change in the animal body before it becomes active. Since salicylic acid can be obtained from dicumarol *in vitro* in practically quantitative yields (392), and of all the compounds studied by Overman *et al.* (313) only those which could yield salicylic acid had anticoagulant action, Link and his associates (235) studied the effect of this acid on prothrombin time. They found that single doses of salicylic acid produced an increase in the prothrombin time of rats maintained on a basal artificial ration low in vitamin K. This anticoagulant action, like that produced by dicumarol, could be prevented by K vitamins and 2-methyl-1,4-naphthoquinone. The activity of salicylic acid was less marked than that of the same amount of dicumarol, and the effect of the K vitamins on the action of the former was much more pronounced.

Rapoport, Wing, and Guest (332) observed that methyl salicylate produced a severe degree of hypoprothrombinemia in rabbits but not in dogs. In rheumatic fever patients receiving salicylic acid or its acetyl derivative, they observed a marked prolongation of the prothrombin time.

Lester (229) was unable to detect salicylates in the urine of rats receiving dicumarol. He also pointed out that this anticoagulant was 25–100 times as effective as salicylic acid, and much more vitamin K was required to prevent its action. Link (234) has suggested that the failure to find the degradation product might not be conclusive, since when salicylic acid was administered to animals only part of it could be recovered in the urine.

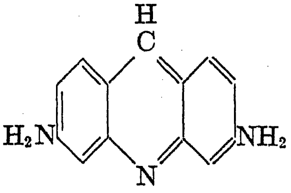
A series of indanedione derivatives of type XI was studied by Kabat, Stohlman, and Smith (192). They found that when R was isobutyl or α-naphthyl, the compounds exerted a marked effect on the prothrombin time of rabbits. Large doses of vitamin K had at best only a very slight effect on the hypoprothrombinemia produced by the isobutyl derivative. When R was ethyl the activity of the product was lower, but the *tert*-butyl derivative was the most active of the group studied—approximately equivalent to dicumarol. Kabat *et al.* considered it unlikely that these indandiones could be degraded to salicylic acid *in vivo*. They also reported that dibenzoylmethane produced a slight hypoprothrombinemia, although it also probably could not be metabolized to salicylic acid in the body.


XI

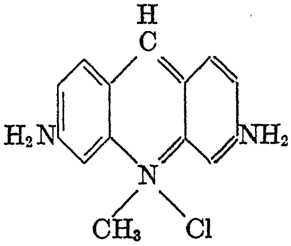
M. Miscellaneous antagonists

Numerous other cases of metabolite antagonists have been reported in addition to those reviewed in the preceding sections. In some instances the number of these studies is too limited to be set apart in separate sections. Other cases are of questionable significance, with respect to either the specificity of the antagonism or the identification of the reversing substance as an essential metabolite. Some of these investigations, which fall more or less within the scope of this review, have been grouped together in this section. It is obviously improbable, however, that all of the substances reported to prevent the biological activity of the diamidines or antimalarials such as quinine and quinacrine are directly related to their mode of action (*cf.* Section IV).

McIlwain (253) found that the growth of bacteria such as *E. coli* and *Strep. hemolyticus*, inhibited by acriflavine or proflavine, could be restored by two types of substances not normally required preformed by the organisms. The first of these was yeast nucleic acid and related substances, such as adenylic acid and cozymase. Yeast nucleic acid was the most effective reversing agent and showed a direct relationship between concentration and effect. The formation of insoluble complexes or salts between proflavine and yeast nucleic acid or adenylic acid was demonstrated. As in the reversal experiments, an excess of



2,8-Diaminoacridine
(proflavine)

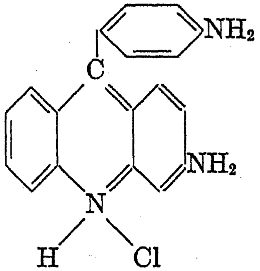


2,8-Diamino-10-methylacridinium
chloride (acriflavine)

nucleic acid was required, suggesting partial dissociation of the salt. Substances of the second type included a mixture of amino acids which was active only over a limited range of acriflavine concentrations. In the presence of this group of substances, but not in their absence, synthetic hydrogen carriers such as methylene blue and riboflavin were effective in further restoring growth. McIlwain considered that the acridine derivatives deprived the organism of the use of certain enzymes or metabolites so that their requirements became more exacting than normal. He suggested that the antibacterial action was due to the combination with and inactivation of enzyme systems of which the nucleotides were essential parts. The amino acids were regarded as substrates or products some of which could be replaced by hydrogen carriers.

Similar results were obtained by Martin and Fisher (281), who showed that the bacteriostatic action of proflavine toward *Staph. aureus* was prevented by adenylic acid and cozymase as well as yeast nucleic acid. No insoluble complexes were formed between proflavine and adenylic acid in saturated aqueous solutions. A number of metabolic intermediates associated with these substances, such as succinic, lactic, pyruvic, and acetic acids, were also reported to interfere with the action of proflavine.

Fitzgerald, Babbitt, and Lee (127, 128) studied the effect of various substances upon the lytic action of bacteriophage on *E. coli*. Of the many types of compounds investigated, 2-amino-5-(*p*-aminophenyl)acridinium chloride (Phosphine GRN), acriflavine, and other acridines were the most active in this respect. Although these derivatives inhibited bacterial growth as well, it was possible to demonstrate marked inhibition of phage multiplication at concentrations of Phosphine GRN which were only slightly bacteriostatic. Moreover, other types



2-Amino-5-(*p*-aminophenyl)acridinium chloride of substances at equally bacteriostatic concentrations failed to prevent the lysis of *E. coli.* It was concluded that the acridines might interfere with some mechanism essential for the reproduction of bacteriophage. Further investigation (128) established the fact that the antiphage effect could be counteracted by yeast nucleic acid. Numerous other substances tested (including vitamins, amino acids, and purines) failed to affect the action of the acridines. The specific nature of the effect of nucleic acid was suggested by studies with acridine-resistant *E. coli.* Strains of this organism made resistant to several of the acridines were still equally susceptible to lysis by bacteriophage. However, the Phosphine GRN-resistant organism was not lysed under the same conditions (i.e., absence of acridine derivative) except in the presence of 10,000 times as large an infecting dose of phage. The presence of nucleic acid again rendered this resistant strain as susceptible to lysis as the original parent strain. Loss of resistance to Phosphine GRN, brought about by many serial passages in a drug-free medium, resulted in the return of normal phage susceptibility.

TABLE 19

*Competition between hydrogen and acridine ions** (*9*)

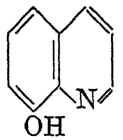
ACRIDINE	INHIBITORY CONCENTRATION OF ACRIDINE IONS $M \times 10$				RATIO OF ACRIDINE TO HYDROGEN IONS			
	pH = 5.7	pH = 6.8	pH = 7.5	pH = 8.3	pH = 5.7	pH = 6.8	pH = 7.5	pH = 8.3
5-Amino...........	1000	130	32	8	510	810	1000	1600
3-Amino...........	740†	57	20		190†	285	641	
2,7-Diamino.......	950	111	25	6.5	500	690	810	1300
3,7-Diamino.......	830†	115	18		210†	580	590	
Average values..	880	103	24	7	352	591	760	1450

* *E. coli* in synthetic medium.

† pH 5.4.

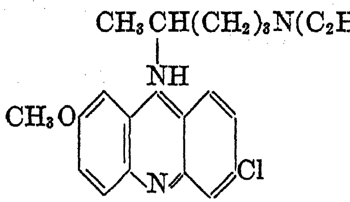
By a comprehensive investigation of over 100 acridine derivatives, Albert, Rubbo, Goldacre, Davey, and Stone (9) demonstrated that the compounds which were 75 per cent or more dissociated at pH 7.3 showed a much greater bacteriostatic activity at that pH than the weak bases. Over a range of hydrogen-ion concentrations in which the degree of ionization of the strong bases did not vary appreciably there was as much as a 100,000-fold change in their minimum effective concentrations. On the other hand, the antibacterial action of the weakly basic acridines did not change greatly with pH. At low pH values (5.4), where all the compounds were practically completely dissociated, both strong and weak bases were essentially equally active. But under conditions of unequal ionization (i.e., at pH 7.3) the activity appeared to be determined by the concentration of cations. Moreover, from pH 5.7 to 8.3, where the hydrogen-ion concentration varies over 400-fold, the ratio of acridine to hydrogen ions was relatively constant (table 19). The authors concluded that their results suggested a competition between acridine ions and hydrogen ions for the same position on a bacterial enzyme surface. They assumed that this position was probably an ionized acidic group, the formation of weakly ionized complexes with the acridine ions inactivating the acidic group in the enzyme.

Combining the knowledge that microörganisms require traces of several metals for growth and that 8-hydroxyquinoline forms undissociated metal complexes, with the known fungistatic action of this compound, Zentmyer (471) reasoned that its effect might be due to a combination with essential metallic ions. Since complex formation does not occur much below pH 3.5, he first demonstrated that the fungistatic action was a function of pH, no effect being observed at low pH values. Zinc was found to be the most important trace element for several fungi, and with these organisms it was shown that the fungistatic action of 8-hydroxyquinoline could be prevented by 1–2 molar equivalents of zinc. With smaller ratios partial growth was obtained, while increasing concentrations of the fungicide were required to inhibit growth in the presence of increasing concentrations of zinc. The author suggested that a zinc–protein enzyme system might be involved, and demonstrated that other substances which form chelate inner complex salts with metals, such as ammonium nitrosophenyl hydroxylamine (Cupferron), also possess fungistatic activity.

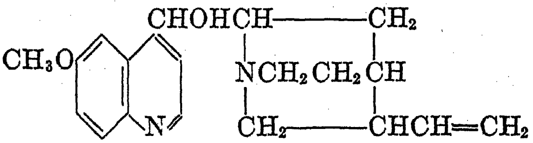


8-Hydroxyquinoline

Silverman and Evans (376, 377) observed that various substances, including spermidine, spermine, trimethylenetetramine, and pantothenic acid, prevented the antibacterial action of quinacrine against *E. coli*. The lesser bacteriostatic action of quinine was also relieved by spermidine. Of the polyamines, spermine was active at a somewhat higher dilution, but spermidine was less toxic to the bacteria. With several soil organisms, it was found that spermidine failed to reverse the action of quinacrine against highly susceptible organisms with exacting growth requirements. However, where the degree of susceptibility to quinacrine and the growth requirements were similar to those of *E. coli*, spermidine prevented the bacteriostatic effect. The authors suggested that spermidine might be an essential metabolite for certain bacteria and that the antibacterial action of quinacrine was due to a competitive inhibition. They were unable to



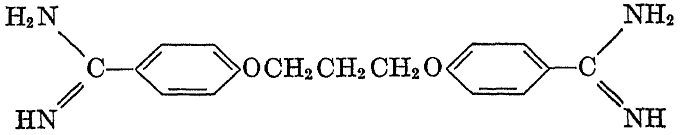
Quinacrine



Quinine

isolate spermine or spermidine from *E. coli*, a result attributed to the minute amounts which might be present. The ability of thiamine, riboflavin, pantothenic acid, glutathione, and nicotinic acid to reverse the action of quinacrine was not considered to be due to specific metabolic effects, since the relative amounts required were much greater

Snell (382) studied the effect of several polyamines (spermidine, trimethylenetetramine, and tetramethylenepentamine) on the antibacterial action of propamidine for *L. casei* and *Strep. fecalis* R. With the former organism only trimethylenetetramine was effective in preventing bacteriostasis at less than growth-inhibitory concentrations. For the latter, spermidine was the most efficient in restoring growth inhibited by propamidine. This effect of the polyamines was evident only over a limited range, and with other bacteria such as *L. arabinosus*, which was susceptible to propamidine only at high concentration, no amount of any of the polyamines was capable of reversing the antibacterial action.



Propamidine

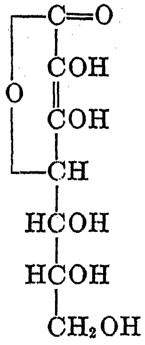
A detailed study of the reversing action of spermine and spermidine was carried out by Miller and Peters (291). They found that the growth-inhibitory action of $M/10{,}000$ quinacrine on *E. coli* in a synthetic medium was never completely reversed by these substances. Growth could be restored to 50 per cent of normal with 2 molar equivalents of spermine or 5 molar equivalents of spermidine. Putrescine was ineffective as a reversing agent. Although the polyamines produced a slight growth stimulation, it appeared to be too small to account for the results. In a complex medium less spermine was required to produce the same degree of reversal. The quinacrine side chain (1-diethylamino-4-aminopentane) showed no effect in either medium. With propamidine ($M/10{,}000$) in a synthetic medium spermine was able to restore growth completely in a ratio of 1:10, while at 1:1 growth was 50 per cent of normal. The authors concluded that spermine and spermidine were probably not acting as essential metabolites because of the high ratios involved.

Other substances have also been reported to prevent the antibacterial action of various diamidines and antimalarials. Thus, Elson (102) found that the bacteriostatic action of propamidine on staphylococci and coli could be prevented by lecithin. Baker, Harrison, and Miller (22) had previously demonstrated a similar effect of lecithins toward various cationic and anionic detergents. Bichowsky (37) noted that the growth-inhibitory effect of 4,4′-diamidinostilbene and 4,4′-diamidinodiphenoxypentane on several microörganisms could be abolished by relatively high concentrations of yeast or animal sodium nucleate. A number of simple substances tested, including adenylic acid, purines, and amino acids, were without effect. It was suggested that the diamidines might act on living cells by combining with the nucleoproteins.

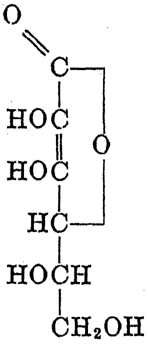
Elson (103) suggested that many of the preceding results might be interpreted as a competitive adsorption between propamidine or other basic antibacterial agents and relatively non-toxic cations, such as the phospholipids and naturally occurring polyamines as well as hydrogen ions, for critical anionic positions on the bacterial cell. Similar considerations were applied to basic dyes by the Stearns (401) and later by Albert and coworkers (6, 7, 9) and Valko and DuBois (416) to other cationic antibacterial agents. It was pointed out by Elson that the protective effect of phosphate ions and nucleic acid might result from the formation of weakly dissociated salts which, in effect, reduced the concentration of toxic cations.

The relation between quinacrine, quinine, propamidine, methylene blue, and riboflavin was examined by Madinaveitia (273), who found that riboflavin prevented the bacteriostatic action of all the other substances when *L. casei* was the test organism. Johnson and Lewin (190) also found that the growth-inhibitory action of quinine on *E. coli*, exhibited over a narrow range of concentration, was reversed by riboflavin as well as by crude cozymase. The latter was the more effective reversing agent, and the authors pointed out that the results were consistent with the view that the effects of quinine on growth and viability might result in part from either a competition or loose combination with cozymase. *In vivo*, Seeler (366) reported that pyridoxine almost completely prevented the antimalarial activity of minimal doses of quinine and quinacrine in blood-induced *P. lophurae* infections in ducks. He suggested the possibility that a competition between the antimalarials and a pyridoxine-like compound for a position in some enzyme system of the parasite might explain this phenomenon.

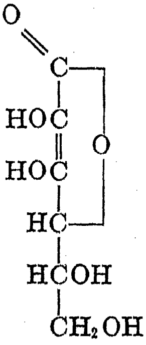
The production of a scurvy-like condition in mice and cotton rats fed glucoascorbic acid (2,3-enediol-*d*-glucoheptono-1,4-lactone) in a purified diet was described by Woolley and Krampitz (455). The harmful action of this substance was not prevented by large amounts of ascorbic acid. However, fresh cabbage or dried grass was effective in counteracting the condition. When 10 per cent of glucoascorbic acid was incorporated in the diet of mice, the resulting symptoms (failure to grow, diarrhea, and hemorrhage) were fatal within two weeks. A majority of the animals survived a 5 per cent diet and eventually showed a gradual return to normal. Closely related substances, such as sodium araboascorbate, did not produce the scurvy-like condition. Unlike mice and cotton rats, which synthesize ascorbic acid, guinea pigs require the preformed vitamin.



Glucoascorbic acid



Ascorbic acid

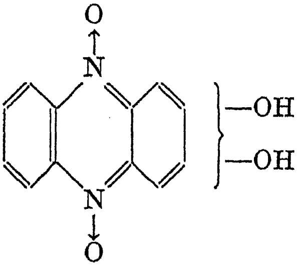


Araboascorbic acid

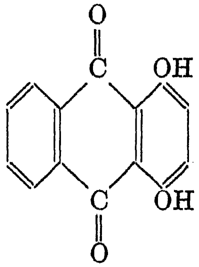
In these animals, Woolley (450) found that glucoascorbic acid produced a scurvy-like condition which could be prevented by suitable quantities of ascorbic acid. He concluded that the results in guinea pigs suggested that the condition could be regarded as an ascorbic acid deficiency in all the animals.

These results were not confirmed by Banerjee and Elvehjem (24), employing rats, chicks, or guinea pigs. Ascorbic acid itself was found to produce similar but less severe symptoms in rats. No hemorrhages were observed, and it was suggested that the failure to grow might be due to the diarrhea. Vitamin C-free liver powder, 2 per cent, with 10 per cent of either glucoascorbic or ascorbic acid allowed normal growth, although the rats showed moderate diarrhea. Similar results were obtained with both chicks and guinea pigs. The authors suggested that the unfavorable effects of glucoascorbic acid might be due, in part, to a modification of the intestinal flora resulting in a deficiency in some factor other than vitamin C which is present in liver powder.

McIlwain (258) investigated the interference of various naphthoquinones and anthraquinones with the bacteriostatic action of iodinin and phenazine di-*N*-oxide. He found that the growth-inhibitory action of the latter substances on streptococci was prevented by relatively small amounts of quinizarin or 2-methyl-1,4-naphthoquinone. The interaction between this latter substance and iodinin appeared to be competitive. A destruction of the *N*-oxides occurred, probably by reduction. As no interaction between iodinin and the quinones occurred in the absence of the organisms, McIlwain concluded that the quinones must have participated to some degree in their metabolism. Although this result could be explained equally well as a function of hydrogen transport, he felt that the structural similarity between the *N*-oxides and quinones suggested that the two classes of compounds functioned at common sites in the organism and that the action of iodinin might be due to its inhibition of systems normally concerned with such quinones.

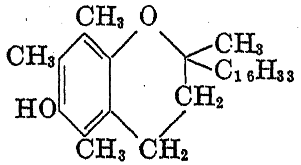


Iodinin

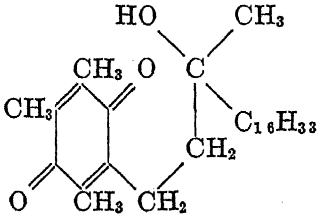


Quinizarin

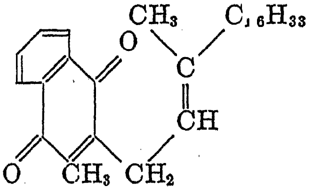
Woolley (453) reported that the administration of α-tocopherol quinone to pregnant mice produced symptoms resembling in some respects an α-tocopherol (vitamin E) deficiency. However, α-tocopherol acetate failed to prevent these symptoms. In addition to the death and resorption of the embryos, excessive vaginal bleeding was observed in the pregnant mice receiving α-tocopherol quinone. The administration of vitamin K_1 overcame both the hemorrhage and the resorption. Equal doses of α-tocopherol quinone were without toxic effects on non-pregnant mice, and the compound did not appear to affect the



α-Tocopherol



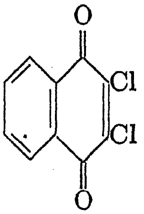
α-Tocopherol quinone



Vitamin K_1

prothrombin time of any of the animals. Since other vitamin K antagonists (*q.v.*) such as dicumarol failed to produce fetal resorption, Woolley concluded that α-tocopherol quinone bore an antagonistic relationship to both vitamin E and vitamin K.

The growth-inhibitory action of 2,3-dichloronaphthoquinone on various microörganisms was examined by Woolley (453a). With organisms such as *Saccharomyces cerevisiae* and *Endomyces vernalis*, 2-methyl-1,4-naphthoquinone or vitamin K_1 prevented the growth inhibition. Over a limited range of concentrations a competitive inhibition appeared to be involved. With other organisms 2-methyl-1,4-naphthoquinone was found to inhibit growth in the same range of concentrations as the dichloro derivative. Consequently, in these cases no reversal could be demonstrated. No vitamin K deficiency could be demonstrated in mice fed purified diets containing 2,3-dichloronaphthoquinone.



2,3-Dichloro-1,4-naphthoquinone

IV. GENERAL CONSIDERATIONS

This review covers a diversified group of antagonists whose physiological actions vary from an effect on the growth of microörganisms to the prevention of a muscular contraction induced by histamine or an influence on the clotting time of blood. The following discussion represents an effort to correlate these apparently unrelated effects on the basis of an underlying similarity in the antagonistic mechanisms involved.

A. Types of antagonism

Direct antagonists may act by interfering with either the synthesis or the utilization (function) of a metabolite. When synthesis is affected, the minimum concentration of metabolite which prevents the action of the antagonist is effective against any concentration of the latter. For example, the minimum daily requirements of thyroxine are effective in preventing the action of any reasonable amount of thiouracil which interferes with its synthesis (19, 270). On the other hand, when a substance affects the utilization or function of a metabolite, a more or less constant ratio usually exists between antagonist and metabolite over a wide range of concentrations. This relationship has been observed in numerous cases, such as the inhibition ratio between sulfanilamide and *p*-aminobenzoic acid (443). As a composite illustration, β-aminobutyric acid inhibits the growth of yeast by interfering with the utilization of β-alanine for the synthesis of pantothenic acid (309). In this case, varying the concentration of β-aminobutyric acid results in a change in the degree of growth inhibi-

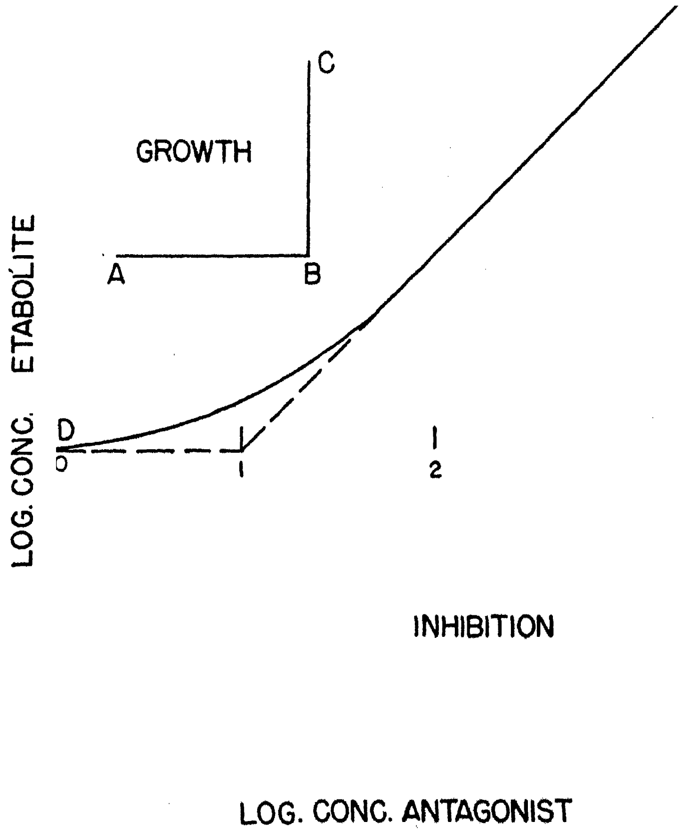


FIG. 3. Different types of antagonism represented by isobol (lines which express conditions giving equal effects; logarithmic scales to base 10). Gaddum (137).

tion in the presence of a normally adequate amount of β-alanine. But the minimum effective concentration of pantothenic acid completely prevents the action of any amount of the antagonist. These generalizations hold, provided amounts which act specifically are not exceeded, for almost any substance will have a non-specific poisoning action on cells if present in sufficiently high concentrations. The differentiation in types of antagonism has been expressed diagrammatically (137) in relation to bacterial growth, as illustrated in figure 3.

In the isobol *ABC* there is no indication of direct antagonism. Here, increasing the concentration of antagonist results in undiminished growth until at *B*, growth is inhibited. However, increasing the concentration of metabolite at this point does not restore growth. *D* represents competitive inhibition of the utilization of a metabolite, where a graded response occurs over a wide range of concentrations. This isobol is drawn through points giving the same degree of growth and shows the more or less constant ratio involved in this type of antagonism. *EFG* indicates that the antagonist affects the synthesis of the metabolite, so that at point *F* increasing the concentration of antagonist no longer affects growth.

Because of the complex and intimate relationships among metabolites in living cells, the distinction between interference with synthesis and interference with utilization can only be applied at a particular stage in any metabolic reaction. A substance which interferes with the utilization of *p*-aminobenzoic acid, for example, probably in turn blocks the synthesis of a more complex metabolite from it. Consequently, more than one metabolite may frequently be found to reverse the biological activity of an antagonist, but only one should show a constant ratio with the antagonist. The sequence in the biochemical synthesis of a complex metabolite E may be represented as follows:

$$\mathrm{A} + \mathrm{B} \xrightarrow[1]{} \mathrm{C}; \qquad \mathrm{C} + \mathrm{D} \xrightarrow[2]{} \mathrm{E}$$

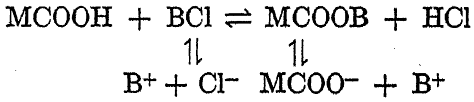
Under certain conditions, an antagonist blocking reaction 1 by a competitive inhibition of the utilization of A to form C could be reversed not only by additional A but also to some extent by B, which would displace reaction 1 toward C, and by D, tending to make up for a deficiency of C in reaction 2. Finally, the presence of E should eliminate the requirement for C altogether, unless this substance also served other metabolic functions or the cell was unable to utilize preformed E. A combination of two antagonists, one of which blocked reaction 1 and the other reaction 2, should be very efficient in preventing the formation of E. There is also the possibility of the existence of another metabolite E^1, able to take over the function of E. This metabolite could then supply an alternative independent route to the same metabolic end point, and so overcome the action of an antagonist which blocked reaction 1 or 2. Under these circumstances, however, there would be no direct relationship between the antagonist and E. It is evident that unless the biochemical or physiological relationships are known, it is frequently difficult to establish direct antagonisms in the absence of a competitive inhibition.

B. *Mechanisms of action*

Direct antagonism involving either the synthesis or the utilization of a metabolite may be due to any one of the following mechanisms: (*1*) salt or complex formation; (*2*) chemical reaction, forming an inactive product; (*3*) oxidation of a metabolite required in the reduced form, or *vice versa;* (*4*) competitive inhibition of an enzyme or tissue receptor associated with a metabolite by a structurally related antagonist.

It is interesting to compare the classification of enzyme inhibitors (153) with those drawn from the fields of pharmacology (69) and chemotherapy (122) which form the basis for the present scheme (*cf.* Section II). As indicated in the Introduction, not all direct antagonists are structurally related to the affected metabolite. However, it is also apparent that the greatest degree of specificity is probably associated with this type of antagonism. In fact, the preceding classification falls more or less in the order of increasing specificity, which is of primary importance to antagonistic mechanisms in living cells.

Salt or complex formation is likely to be relatively non-specific, except in certain instances where an antagonist forms a specific insoluble or undissociated complex with a metabolite such as one of the essential trace metals. More frequently, the antagonists which act by this mechanism are organic acids or bases or heavy metals which form salts with metabolites of opposite charge. For example, the antibacterial action of the organic bases may be represented as a simple double decomposition reaction as indicated:



$$\begin{array}{cccc} \mathrm{MCOOH} + & \mathrm{BCl} \rightleftharpoons & \mathrm{MCOOB} & + \mathrm{HCl} \\ & \upharpoonleft\downharpoonright & \upharpoonleft\downharpoonright & \\ & \mathrm{B^+ + Cl^-} & \mathrm{MCOO^- + B^+} & \end{array}$$

In this equation M represents the residue of an acidic metabolite and B^+ the cation of an organic base, the action of which depends on the dissociation constants of BCl and MCOOB. Since there are numerous acidic and basic metabolites, including proteins, the problem of determining the specific metabolite involved is frequently gratuitous. Several different metabolites may be found to reverse the same antagonist (*cf.* Section III,M), and relatively non-toxic ions of the same or opposite charge which are not metabolites have been shown to act in the same manner (416). Many instances of the so-called "interference phenomenon" (460) or chemotherapeutic interference (125) can probably be explained by a mechanism similar to that demonstrated for the protective action of relatively non-toxic ions (416). Consequently, in most cases of antagonisms involving salt formation, it can only be concluded that a competition for hydrogen or hydroxyl ions (ion exchange) is involved.

Antagonists which combine chemically with metabolites to form inactive products, and those which affect oxidation–reduction systems, form an intermediate group with respect to their specificity of action. In the former category, the effects may sometimes be limited to a particular chemical grouping such as the mercapto group (*cf.* Section III,D). Since there are numerous —SH-containing essential metabolites, however, it is usually not possible to demonstrate complete specificity. Nevertheless, the action of trivalent arsenicals and some of the antibiotics like clavicin can be explained most readily through their inactivation of vital mercapto groups. On the other hand, any antagonist which reacts with mercapto compounds may be reversed by metabolites containing this grouping, whether or not this mechanism explains its biological activity, and regardless of whether the —SH compounds are actually metabolites. As a result, special care should be taken in interpreting the observations with highly reactive substances such as penicillin. All substances which inactivate this antibiotic obviously cannot form the basis for an explanation of its mode of action. To a considerable extent this type of difficulty is inherent in all mechanisms involving a chemical reaction, although this approach may be of value in certain cases when the metabolite contains unique chemical groupings. Otherwise, the effects are frequently too non-specific to be of general interest in living cells.

Many oxidizing agents have antibacterial activity which is relatively non-specific with respect to the affected metabolite. Within narrow limits of potential, it is possible that more specific effects involving an oxidation or reduction mechanism may be found. For example, although the mechanism of action of thiouracil is not well understood at present, it is evident that an oxidation of iodide ion to organic iodine is essential for the synthesis of thyroxine. By reducing any free iodine as rapidly as it is formed, thiouracil may effectively prevent the formation of organic iodine (295). It is also possible, of course, that thiouracil inhibits an enzyme system associated with the conversion of iodide ion to organic iodine by some other mechanism. In any event, it is evident that in the absence of any apparent structural relationship, a highly specific action on the synthesis of thyroxine is found in the thiouracil-type compounds.

Under different circumstances, the same type of antagonist, e.g., heavy metals, may act through divergent mechanisms. For instance, auric ions can form insoluble salts with acidic metabolites or essential —SH groups, but they can also oxidize the latter to inactive disulfides. Consequently, depending on the conditions, the mechanism of action of gold salts may involve salt formation or oxidation.

The action of all of the foregoing types of antagonists appears to involve a competitive mechanism. Thus, the formation of undissociated salts may be regarded as a competition for hydrogen or hydroxyl ions; the arsenicals may compete for vital mercapto groups; and possibly thiouracil competes with a metabolite for iodine. In all cases a definite ratio can usually be demonstrated, although the range of concentrations for thiouracil is rather narrow (16), owing possibly to the limited oxidative capacity of the thyroid gland.

The distinguishing feature of the other class of competitive antagonists—namely, the close structural relationship between antagonist and metabolite—probably accounts for the high degree of specificity associated with their biological activity. This specificity allows the isolation of a single metabolic process which might otherwise be impossible under the complex conditions occurring in living cells.

The mechanism underlying the action of antagonists structurally related to metabolites is based on studies with isolated enzyme systems (*cf.* Section II,A). Similar phenomena are found in the cross-reactions and the effect of haptens in the field of immunology (217, 276). The metabolite and antagonist are assumed to compete for an active center or receptor in which there are polar groups oriented in such a manner as to exert a strong, specific attractive force on the metabolite. These forces, involving both primary and secondary valencies, are probably similar to those postulated for immunological reactions (317). Because of the similarity in the nature and steric configuration of the active groupings of the metabolite and the antagonist, both are presumably attracted to the same active center, but the antagonist is unable to carry out the normal physiological function of the metabolite because of slight differences in one of the essential groupings.

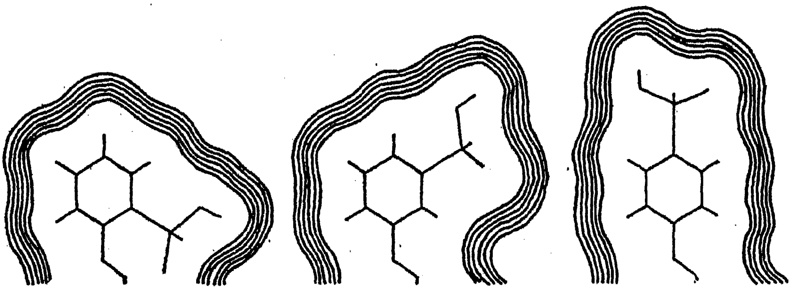


FIG. 4. Antibody cavities specific to *o*-, *m*-, and *p*-azophenylarsonic acid haptenic groups, respectively. (Scale drawings with circumferential contours corresponding to close fit; radial dilatation in increments of 0.2 Å.) Pauling and Pressman (317).

While the exact nature of the active centers or receptors is unknown, they may be pictured as cavities in a complex molecule similar to those suggested for hapten groupings in antibody molecules (figure 4). If one assumes that superimposed on steric configuration there are polar groups at appropriate points which bind the metabolite to the surface of the cavity, the high degree of specificity of both metabolite and antagonist can be accounted for.

The relative attractive forces operating between the metabolite and the active centers, compared with the attraction of the antagonist to the active centers, will determine the efficiency of the antagonist. This efficiency is reflected in the molar inhibition ratio between antagonist and metabolite. With few exceptions, these ratios are greater than unity, because the firmness of the binding of the metabolite to the receptor is usually greater than the binding between the antagonist and the receptor.

From pharmacological studies with acetylcholine and epinephrine Gaddum (136) has shown that the quantitative data for many competitive antagonisms can be interpreted by a simple equation. If *M* is the concentration of metabolite, and A the concentration of antagonist competing for the same receptors, and m and a the corresponding proportions of the receptors occupied by each, so that $(1 - m - a)$ is the proportion of free receptors, then for equilibrium:

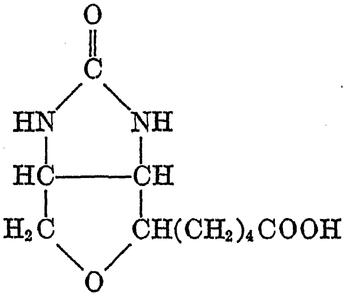
$$K_1M(1 - m - a) = m, \text{ and } K_2A(1 - m - a) = a \quad .$$

Eliminating a gives:

$$K_1M = (1 + K_2A)\frac{m}{1 - m} \tag{1}$$

When $A = 0$, this expression becomes the simple mass action equilibrium. Equation 1 is applicable so long as the reaction is of the first order. Actually, in certain cases the reaction does not appear to be of the first order, although this observation may be related to the complexity of these phenomena in living cells (137).

Table 20 illustrates a number of examples of structurally related competitive metabolite antagonists which act as growth inhibitors. It is doubtful, however, that one should conclude from such results that substituting the same groupings in other metabolites will lead to competitive antagonists in all cases. For instance, although replacing the sulfur atom in methionine by oxygen produces a competitive antagonist (341), a corresponding change in the biotin molecule results in a compound, oxybiotin, with the same type of biological activity as biotin itself (95, 178, 178a, 323a, 357, 358). Similarly, the replacement of the carboxyl group in anthranilic acid by a sulfonic acid group leads to an inert compound with respect to the antagonism of anthranilic acid (381). The evidence drawn from more extensive studies with isolated enzymes also suggests that each system may exhibit its own peculiarities, and structural changes which produce effective antagonists for one system may not produce the same results in another case.

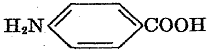
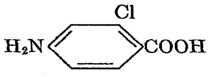
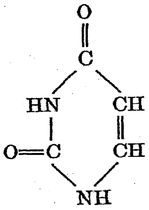
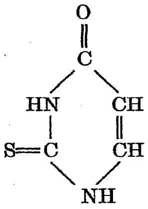
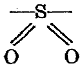
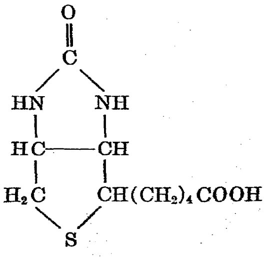
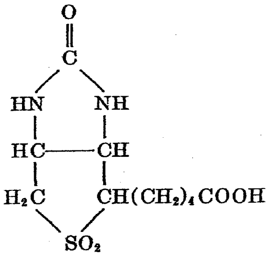

Oxybiotin

C. *Applications and conclusions*

The intimate relation of enzymes to life processes makes them the logical starting point for a discussion of the application of antagonists in living cells. Wherever possible, the study of antagonists with isolated enzyme systems should simplify many of the problems encountered under more complex conditions. It

TABLE 20

Structurally related competitive metabolite antagonists

ATOM OR GROUP REPLACED	REPLACING ATOM OR GROUP	EXAMPLE		REFERENCE
		Metabolite	Antagonist	
—H	—Cl	 *p*-Aminobenzoic acid	 4-Amino-2-chlorobenzoic acid	Wyss *et al.* (464)
—H	—CH_3	$H_2NCH_2CH_2COOH$ β-Alanine	$H_2NCH(CH_3)CH_2COOH$ β-Aminobutyric acid	Nielson (306)
=O	=S	 Uracil	 2-Thiouracil	Roepke and Jones (347)
—S—	—O—	$CH_3SCH_2CH_2CH(NH_2)COOH$ Methionine	$CH_3OCH_2CH_2CH(NH_2)COOH$ Methoxinine	Roblin *et al.* (341)
—S—		 Biotin	 Biotin sulfone	Dittmer and du Vigneaud (85)

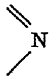
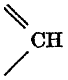
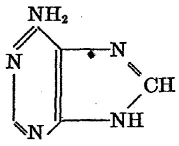
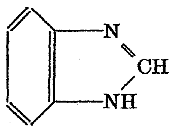
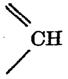
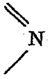
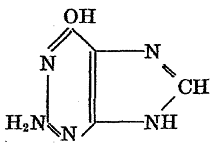
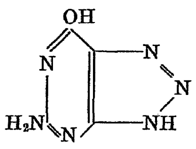
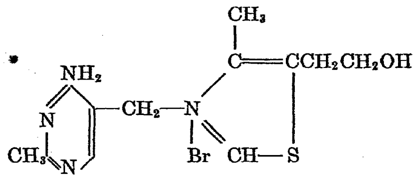
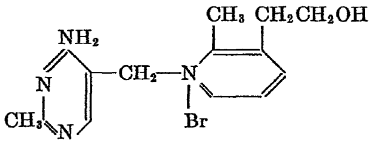
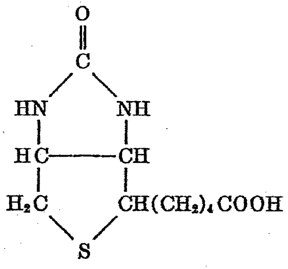
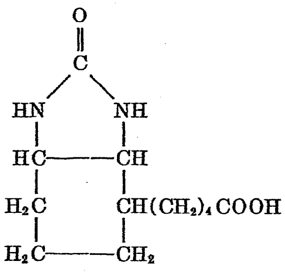
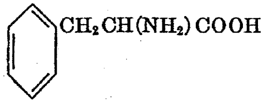
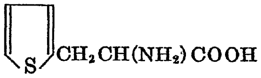
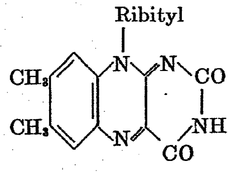
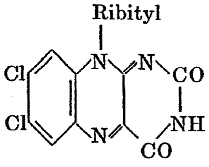
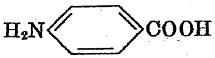
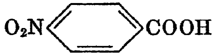
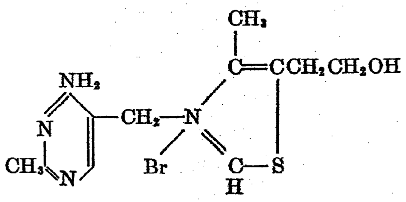
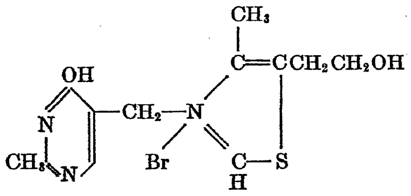
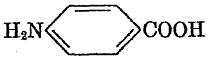
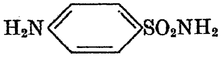
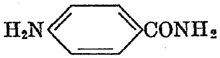
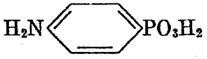
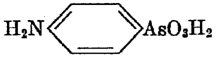
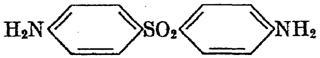
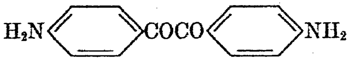
		 Adenine	 Benzimidazole	Woolley (447)
		 Guanine	 5-Amino-7-hydroxy-1-*v*-triazolo[*d*]pyrimidine	Roblin *et al.* (341)
—S—	—CH=CH—	 Thiamine	 Pyrithiamine	Robbins (339) Woolley and White (457, 458)
—S—	$—CH_2—CH_2—$	 Biotin	 δ-(2,3-Ureylenecyclohexyl)valeric acid	English *et al.* (108)

TABLE 20—*Continued*

Structurally related competitive metabolite antagonists

ATOM OR GROUP REPLACED	REPLACING ATOM OR GROUP	EXAMPLE		REFERENCE
		Metabolite	Antagonist	
—CH=CH—	—S—	 Phenylalanine	 β-2-Thienylalanine	du Vigneaud *et al.* (420)
$—CH_3$	—Cl	 Riboflavin	 Chloroflavin	Kuhn *et al.* (209)
$—CH_3$	$—C_2H_5$	$CH_3SCH_2CH_2CH(NH_2)COOH$ Methionine	$C_2H_5SCH_2CH_2CH(NH_2)COOH$ Ethionine	Dyer (96) Harris and Kohn (159)
$—NH_2$	$—NO_2$	 *p*-Aminobenzoic acid	 *p*-Nitrobenzoic acid	Miller (292)
$—NH_2$	—OH	 Thiamine	 Oxythiamine	Soodak and Cerecedo (388)

—COOH	—CH_2OH	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2COOH$ Pantothenic acid	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CH_2OH$ Pantothenyl alcohol	Snell and Shive (386)
—COOH	—COC_6H_5	Pantothenic acid	$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2COC_6H_5$ Phenyl pantothenone	Woolley and Collyer (454)
—COOH	—SO_3H	$(CH_3)_2CHCH(NH_2)COOH$ Valine	$(CH_3)_2CHCH(NH_2)SO_3H$ α-Aminoisobutanesulfonic acid	McIlwain (252)
—COOH	—SO_2NH_2	 *p*-Aminobenzoic acid	 Sulfanilamide	Woods (443)
—COOH	—$CONH_2$	*p*-Aminobenzoic acid	 *p*-Aminobenzamide	Hirsch (175)
—COOH	—PO_3H_2	*p*-Aminobenzoic acid	 Phosphanilic acid	Kuhn *et al.* (206)
—COOH	—AsO_3H_2	*p*-Aminobenzoic acid	 Arsanilic acid	Peters (319)
—COOH	—SO_2—	*p*-Aminobenzoic acid	 *p,p′*-Diaminodiphenyl sulfone	Levaditi and Perault (231)
—COOH	—COCO—	*p*-Aminobenzoic acid	 *p,p′*-Diaminobenzil	Kuhn *et al.* (206)

cannot be arbitrarily concluded, however, that because substances inhibit certain enzymes they function by the same mechanisms in living cells.

The examples in table 1 (Section II) indicate a few of the numerous inhibitors which have been studied with isolated enzymes. Unfortunately, many of the enzymes involved in the utilization of simple metabolites are unknown, particularly those associated with anabolic reactions. This situation led Kuhn *et al.* (210) to reverse the usual procedure and calculate the dissociation constant of the hypothetical enzyme associated with the utilization of pantothenic acid from data obtained with living cells. Such an application may be somewhat speculative in nature, but it illustrates the versatility of the metabolite antagonist approach.

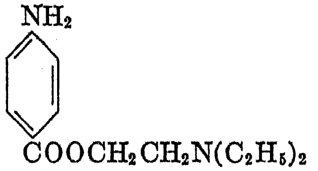
A number of respiratory enzymes have been studied extensively, and the relation of the simple metabolites to the holoenzymes established. These systems are frequently vital to all cells so that, from the chemotherapeutic point of view, antagonists affecting respiratory processes appear to offer fewer possibilities because of their potential toxicity. The rapidly fatal effect of pyrithiamine for higher animals (457) demonstrates the toxic action of this antagonist in mammalian cells dependent on an external source of thiamine. On the other hand, much valuable information in the fields of animal and bacterial nutrition can be obtained with this type of antagonist.

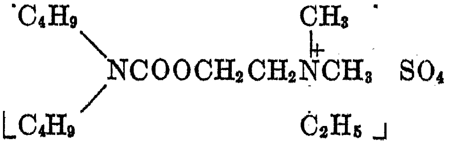
Because the rate of growth and of multiplication of host cells is probably much slower than that of pathogenic organisms, antagonists which affect anabolic reactions seem to offer greater promise as chemotherapeutic agents. The sulfanilamides are, in all likelihood, an outstanding example of this type of antagonism. The arsenicals, on the other hand, probably owe their lower toxicity to the host to the fact that they are concentrated in the invading parasites (166, 467).

One of the complicating factors in the application of metabolite antagonists to chemotherapy is the presence of too high a concentration of some metabolites in the body fluids and tissues of higher animals and man. This difficulty was encountered by McIlwain and Hawking (264), who were able to protect rats but not mice from experimental streptococcic infections with pantoyltaurine because of the lower concentration of pantothenic acid in the body fluids of rats. In any case, the pharmacological properties of pantoyltaurine would probably limit its usefulness as a chemotherapeutic agent. However, other pantothenic acid antagonists have been found which are highly effective in mice (433, 437) and may prove to be of value in chemotherapy. As Welch (428) pointed out, the study of antagonists of metabolites which occur only in minute amounts in body fluids is most likely to lead to practical applications in chemotherapy.

There is also another phenomenon which is of theoretical interest and at the same time limits the application of competitive inhibitions of bacterial growth to chemotherapy; that is, the susceptibility of various organisms to antagonists of metabolites required preformed, compared with those which they are capable of synthesizing. In several instances, such as *p*-aminobenzoic acid and purine antagonists, the growth of practically all microörganisms appears to be inhibited regardless of their requirements. Antagonists of other metabolites, e.g., biotin, pantothenic acid, and thiamine, seem to inhibit the growth of only those organisms which require the preformed metabolite. Analogous observations have been made with isolated enzyme systems (*cf.* table 1), and the explanation may rest on a difference in the manner in which various organisms utilize the particular metabolite. A simpler explanation can be based on the observation that certain closely related analogues may actually serve as intermediates or be degraded to yield intermediates which can be utilized by the organisms for the synthesis of the metabolite. Under these circumstances, only organisms lacking the synthetic ability can be expected to be sensitive to this type of antagonist. Evidence for this explanation has been presented for biotin sulfone (86) and desthiobiotin (87, 408a), *p*-nitrobenzoic acid (292), pantoyltaurine (394), and pyrithiamine (339, 449).

The pharmacological action of several drugs has been accounted for on the basis of enzyme inhibitions (Section II), and antagonists have been found which block the action of acetylcholine, epinephrine, and histamine in isolated tissues or intact animals. Clark (69) explained the action of direct antagonists on the basis of a union with specific receptors. Similarly, Thimann (412) suggested that the action of many local anesthetics such as procaine and cocaine might be due to their structural resemblance to acetylcholine. He assumed that nerve stimulation is brought about by a combination of acetylcholine with receptors, which may resemble the active centers of enzymes. By competing with the metabolite for the receptors, the local anesthetics are assumed to block the nerve stimulation and so produce anesthesia. Both procaine (165) and dibutoline (320) have been shown to prevent acetylcholine-induced contractions in certain types of muscle tissue.[17]


Procaine


Dibutoline

The relation of structure to pharmacological action is sometimes complicated by the observation that different members of a closely related series produce opposite pharmacological effects. This enigma may be resolved in certain of those cases which involve the antagonism of a metabolite normally destroyed by an enzyme system. If the drugs act both as direct antagonists and as enzyme inhibitors, the net effect of any member of the group will depend on its predominant action. Thus, the primary effect of one substance, A_1, may be to inhibit the enzyme system which destroys the metabolite. Because it protects the metabolite from destruction, its action will be like that of the metabolite. But the predominant effect of another member of the series, A_2, may be a direct antagonism of the metabolite so that its pharmacological action will be the opposite of A_1.

---

[17] Using leech muscle, Hazard, Corteggiani, and Pelou (167a) did not observe any antagonism of acetylcholine with procaine. In fact, the local anesthetic appeared to sensitize the muscle to acetylcholine.

It should be emphasized again that antagonisms in isolated tissues or intact animals may be exceedingly complex. For instance, ephedrine in certain cases enhances the action of epinephrine, presumably by decreasing the rate of hormone destruction (138), but under the same conditions higher concentrations of ephedrine are antagonistic (362). The antagonistic effect of ergotoxine toward the action of epinephrine on the rabbit's ileum is very pronounced, but it does not noticeably affect the latter's action on the rabbit's colon (69). Atropine, an acetylcholine antagonist, also counteracts some of the actions of epinephrine. Moreover, epinephrine and acetylcholine themselves can produce opposite effects in the same tissue, and so neutralize each other. Finally, the antagonists are usually not without some pharmacological activity of their own. Thus, diverse tissues may respond differently to the same stimulus, and one may assume that variations in the pattern of receptors determine both the type and intensity of pharmacological response and antagonistic action (69).

The mechanisms of action of all drugs obviously cannot be explained on the basis of a direct antagonism. Nevertheless, it is apparent that simple enzymatic reactions, the growth of microörganisms, and the function or response of various tissues may be antagonized by mechanisms which are strikingly similar. Consequently, it may not be unreasonable to assume that this resemblance is more than superficial and that, for example, the inhibitory action of malonic acid on succinic dehydrogenase, the antibacterial action of pantoyltaurine, and the antagonistic effect of trimethyloctylammonium chloride toward the action of acetylcholine on smooth muscle are closely related phenomena. In each case the antagonist appears to compete with the metabolite for an active center or receptor and in so doing block the normal reaction, whether it be the oxidation of succinic acid, the growth of a microörganism dependent on pantothenic acid, or the contraction of a muscle under the stimulus of acetylcholine.

Since in many respects it is a relatively new and rapidly developing field, it is not possible to assess all the implications inherent in the broad concept of metabolite antagonists. However, as an approach to the mechanism of action of a number of drugs, as a guide in the synthesis of new therapeutic agents, and as a means of evaluating the normal mode of synthesis and function of metabolites in living cells, the concept appears to offer many possibilities as yet unexplored.

It is a pleasure to acknowledge my indebtedness to my wife and to many of the members of the Chemotherapy Division in these Laboratories for their assistance in reading and criticizing various parts of this manuscript and for their help and encouragement during its preparation.

V. REFERENCES

(1) Ackermann, D.: Naturwissenschaften **27,** 515 (1939).
(2) Ackermann, D., and Mauer, H.: Arch. ges. Physiol. (Pflüger's) **247,** 450 (1944); Chem. Abstracts **39,** 3803 (1945).
(3) Ackermann, D., and Wasmuth, W.: Z. physiol. Chem. **259,** 28 (1939).
(4) Ackermann, D., and Wasmuth, W.: Z. physiol. Chem. **260,** 155 (1939).
(5) Aeschlimann, J. A., and Reinert, M.: J. Pharmacol. **43,** 413 (1931).
(6) Albert, A.: Lancet **1942, II,** 633.
(7) Albert, A.: Australian J. Sci. **6,** 137 (1944).
(8) Albert, A., Falk, J. E., and Rubbo, S. D.: Nature **153,** 712 (1944).
(9) Albert, A., Rubbo, S. D., Goldacre, R. J., Davey, M. E., and Stone, J. D.: Brit. J. Exptl. Path. **26,** 160 (1945).
(10) Anderson, E. G., Pilgrim, F. J., and Elvehjem, C. A.: Proc. Soc. Exptl. Biol. Med. **55,** 39 (1944).
(11) Anderson, G. W., Halverstadt, I. F., Miller, W. H., and Roblin, R. O., Jr.: J. Am. Chem. Soc. **67,** 2197 (1945).
(12) Anderson, T. F.: Science **101,** 565 (1945).
(13) Armstrong, E. F.: Proc. Roy. Soc. (London) **73,** 516 (1904).
(14) Astwood, E. B.: J. Am. Med. Assoc. **122,** 78 (1943).
(15) Astwood, E. B.: J. Pharmacol. **78,** 79 (1943).
(16) Astwood, E. B.: *Harvey Lectures.* Science Press Printing Company, Lancaster, Pennsylvania (1945).
(17) Astwood, E. B., and Bissell, A.: Endocrinology **34,** 282 (1944).
(18) Astwood, E. B., Bissell, A., and Hughes, A. M.: Endocrinology, in press.
(19) Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R.: Endocrinology **32,** 210 (1943).
(20) Atkinson, N., and Stanley, N. F.: Australian J. Exptl. Biol. Med. Sci. **21,** 249, 255 (1943).
(21) Auhagen, E.: Z. physiol. Chem. **274,** 48 (1942).
(22) Baker, Z., Harrison, R. W., and Miller, B. F.: J. Exptl. Med. **74,** 621 (1941).
(23) Balls, A. K., and Köhler, F.: Ber. **64,** 34 (1931).
(24) Banerjee, S., and Elvehjem, C. A.: Proc. Soc. Exptl. Biol. Med. **60,** 4 (1945).
(25) Barnett, J.: J. Chem. Soc. **1944,** 5.
(26) Barnett, J., Dupré, D. J., Holloway, B. J., and Robinson, F. A.: J. Chem. Soc. **1944,** 94.
(27) Barnett, J. W., and Robinson, F. A.: Biochem. J. **36,** 357, 364 (1942).
(28) Barron, E. S. G., and Singer, T. P.: Science **97,** 356 (1943).
(29) Barron, E. S. G., and Singer, T. P.: J. Biol. Chem. **157,** 221 (1945).
(30) Baumann, E. J., Metzger, N., and Marine, D.: Endocrinology **34,** 44 (1944).
(31) Behrens, O. K.: J. Biol. Chem. **141,** 503 (1941).
(32) Bell, P. H., and Roblin, R. O., Jr.: J. Am. Chem. Soc. **64,** 2905 (1942).
(33) Bergel, F., and Todd, A. R.: J. Chem. Soc. **1937,** 1504.
(34) Bernheim, F.: *The Interaction of Drugs and Cell Catalysts.* Burgess Publishing Company, Minneapolis, Minnesota (1942).
(35) Bernheim, F., and Bernheim, M. L. C.: Cold Spring Harbor Symposia Quant. Biol. **7,** 174 (1939).
(36) Best, C. H., and McHenry, E. W.: Physiol. Rev. **11,** 371 (1931).
(37) Bichowsky, L.: Proc. Soc. Exptl. Biol. Med. **57,** 163 (1944).
(38) Blanchard, K. C.: J. Biol. Chem. **140,** 919 (1941).
(39) Blanchard, K. C.: Personal communication.
(40) Blaschko, H., Richter, D., and Schlossmann, H.: Biochem. J. **31,** 2187 (1937).
(41) Bliss, E. A., and Long, P. H.: Bull. Johns Hopkins Hosp. **69,** 14 (1941).
(42) Bloch, H., and Erlenmeyer, H.: Helv. Chim. Acta **25,** 694, 1062 (1942).

(43) Bonner, D., Tatum, E. L., and Beadle, G. W.: Arch. Biochem. **3,** 71 (1943).

(44) Bourque, J. E., and Loew, E. R.: Am. J. Physiol. **138,** 341 (1943).

(45) Bovet, D., Horclois, R., and Fournel, J.: Compt. rend. soc. biol. **138,** 165 (1944).

(46) Bovet, D., Horclois, R., and Walthert, F.: Compt. rend. soc. biol. **138,** 99 (1944).

(47) Bovet, D., and Staub, A. M.: Compt. rend. soc. biol. **124,** 547 (1937).

(48) Brackett, S., Waletzky, M., and Baker, M.: To be published.

(49) Braun, H., and Schaeffer, H.: Z. Hyg. Infektionskrank. **89,** 339 (1919).

(50) Brodie, D. C., Hiestand, W. A., and Jenkins, G. L.: J. Am. Pharm. Assoc. **34,** 73 (1945).

(51) Brueckner, A. H.: Yale J. Biol. Med. **15,** 813 (1943).

(52) Buchman, E. R., Heegaard, E., and Bonner, J.: Proc. Natl. Acad. Sci. U. S. **26,** 561 (1940).

(53) Burchell, H. B., and Varco, R. L.: J. Pharmacol. **75,** 1 (1942).

(54) Burtner, R. R., and Cusic, J. W.: J. Am. Chem. Soc. **65,** 262 (1943).

(55) Burtner, R. R., and Cusic, J. W.: J. Am. Chem. Soc. **65,** 1582 (1943).

(56) Bywater, W. G., McGinty, D. A., and Jenesel, N. D.: J. Pharmacol. **85,** 14 (1945).

(57) Campbell, D., Landgrebe, F. W., and Morgan, T. N.: Lancet **1944, II,** 630.

(58) Campbell, H. A., and Link, K. P.: J. Biol. Chem. **138,** 21 (1941).

(59) Cavallito, C. J., and Bailey, J. M.: Science **100,** 390 (1944).

(60) Cavallito, C. J., Bailey, J. M., Haskell, T. H., McCormick, J. R., and Warner, W. F.: J. Bact. **50,** 61 (1945).

(61) Chambon, M., Lavaud, M. T., and Martin, P.: Compt. rend. soc. biol. **137,** 469 (1943).

(62) Chapman, C. J.: Quart. J. Pharm. Pharmacol. **17,** 314 (1944).

(63) Chauchard, B., and Chauchard, P.: Compt. rend. soc. biol. **137,** 708 (1943).

(64) Chen, G., Geiling, E. M. K., and MacHatton, R. M.: J. Infectious Diseases **76,** 152 (1945).

(65) Chick, H.: J. Hyg. **8,** 92 (1908).

(66) Chow, B. F., and McKee, C. M.: Proc. Soc. Exptl. Biol. Med. **58,** 175 (1945).

(67) Clark, A. J.: J. Physiol. **61,** 547 (1926).

(68) Clark, A. J.: *The Mode of Action of Drugs on Cells.* Williams & Wilkins Company, Baltimore (1933).

(69) Clark, A. J.: Heffter's *Handbuch der experimentellen Pharmakologie,* Ergänz. 4. J. Springer, Berlin (1937).

(70) Clarke, H. T.: J. Am. Chem. Soc. **66,** 652 (1944).

(71) Climenko, D. R., Homburger, E., and Messer, F. H.: J. Lab. Clin. Med. **27,** 289 (1941).

(72) Cohen, A., King, H., and Strangeways, W. I.: J. Chem. Soc. **1931,** 3043.

(73) Cohen, A., King, H., and Strangeways, W. I.: J. Chem. Soc. **1932,** 2505.

(74) Colwell, C. A., and McCall, M.: Science **101,** 592 (1945).

(75) Controulis, J., and Banks, C. K.: J. Am. Chem. Soc. **67,** 1946 (1945).

(76) Coombs, H. I.: Biochem. J. **21,** 1259 (1927).

(77) Couceiro, A., Vieira, L. G., and Moraes, J. de: Rev. brasil. biol. **4,** 173 (1944); Chem. Abstracts **39,** 350 (1945).

(78) Cowles, P. B.: Yale J. Biol. Med. **14,** 599 (1942).

(79) Cromer, H. E., Jr., and Barker, N. W.: Proc. Staff Meetings Mayo Clinic **19,** 217 (1944).

(80) Davidson, C. S., and MacDonald, H.: New Engl. J. Med. **229,** 353 (1943).

(81) Dempsey, E. W.: Endocrinology **34,** 27 (1944).

(82) Denkewalter, R., Cook, M. A., and Tishler, M.: Science **102,** 12 (1945).

(83) DeRobertis, E., and Gonçalves, J. M.: Endocrinology **36,** 245 (1945).

(84) Dimond, N. S.: Science **94,** 420 (1941).

(85) Dittmer, K., and du Vigneaud, V.: Science **100,** 129 (1944).

(86) Dittmer, K., du Vigneaud, V., György, P., and Rose, C. S.: Arch. Biochem. **4**, 229 (1944).

(87) Dittmer, K., Melville, D. B., and du Vigneaud, V.: Science **99**, 203 (1944).

(88) Dixon, T. F.: Nature **153**, 289 (1944).

(89) Doermann, A. H.: Arch. Biochem. **5**, 373 (1944).

(90) Dorfman, A., and Koser, S. A.: J. Infectious Diseases **71**, 241 (1942).

(91) Dorfman, A., Rice, L., Koser, S. A., and Saunders, F.: Proc. Soc. Exptl. Biol. Med. **45**, 750 (1940).

(92) Doudoroff, M.: Proc. Soc. Exptl. Biol. Med. **53**, 73 (1943).

(93) Douglas, C. G., Haldane, J. S., and Haldane, J. B. S.: J. Physiol. **44**, 275 (1912).

(94) Dubos, R. J.: J. Am. Med. Assoc. **124**, 633 (1944).

(95) Duschinsky, R., Dolan, L. A., Flower, D., and Rubin, S. H.: Arch. Biochem **6**, 480 (1945).

(96) Dyer, H. M.: J. Biol. Chem. **124**, 519 (1938).

(97) Eagle, H.: J. Pharmacol. **66**, 436 (1939).

(98) Eakin, R. E., Snell, E. E., and Williams, R. J.: J. Biol. Chem. **140**, 535 (1941).

(99) Edlbacher, S., Baur, H., and Becker, M.: Z. physiol. Chem. **265**, 61 (1940).

(100) Edlbacher, S., Jucker, P., and Baur, H.: Z. physiol. Chem. **247**, 63 (1937).

(101) Ellis, F. W.: Federation Proc. **4**, 117 (1945).

(102) Elson, W. O.: J. Biol. Chem. **154**, 717 (1944).

(103) Elson, W. O.: J. Infectious Diseases **76**, 193 (1945).

(104) Elvehjem, C. A., and Tepley, L. J.: Chem. Rev. **33**, 185 (1943).

(104a) Emerson, G. A., and Soughwick, P.: J. Biol. Chem. **160**, 169 (1945).

(105) Emerson, G. A., and Tishler, M.: Proc. Soc. Exptl. Biol. Med. **55**, 184 (1944).

(105a) Emerson, G. A., Wurtz, E., and Johnson, O. H.: J. Biol. Chem. **160**, 165 (1945).

(106) Emmelin, N., Kahlson, G. S., and Lindström, K.: Acta Physiol. Scand. **3**, 39 (1941).

(107) Englehardt, E., and Loewi, O.: Arch. exptl. Path. Pharmakol. **150**, 1 (1930).

(108) English, J. P., Clapp, R. C., Cole, Q. P., Halverstadt, I. H., Lampen, J. O., and Roblin, R. O., Jr.: J. Am. Chem. Soc. **67**, 295 (1945).

(109) English, J. P., Clark, J. H., Clapp, J. W., Seeger, D., and Ebel, R. H.: J. Am. Chem. Soc., in press.

(110) Erhlich, P.: Ber. **42**, 17 (1909).

(111) Erlenmeyer, H., Bloch, H., and Kiefer, H.: Helv. Chim. Acta **25**, 1066 (1942).

(112) Erlenmeyer, H., and Würgler, W.: Helv. Chim. Acta **25**, 249 (1942).

(113) Euler, H. v.: Ber. **75**, 1876 (1942).

(114) Euler, H. v., and Josephson, K.: Z. physiol. Chem. **157**, 122 (1926).

(115) Euler, H. v., and Ahlström, L.: Z. physiol. Chem. **279**, 176 (1943).

(116) Fantl, P.: Australian J. Sci. **6**, 23 (1943).

(117) Feeney, R. E., and Strong, F. M.: J. Am. Chem. Soc. **64**, 881 (1942).

(118) Feldberg, W.: Ann. Rev. Physiol. **3**, 671 (1941).

(119) Feldt, A.: Klin. Wochschr. **20**, 945 (1941).

(120) Field, J. B., Larsen, G. E., Spero, L., and Link, K. P.: J. Biol. Chem. **156**, 725 (1944).

(121) Fildes, P.: Biochem. J. **32**, 1600 (1938).

(122) Fildes, P.: Lancet **1940, I**, 955.

(123) Fildes, P.: Brit. J. Exptl. Path. **21**, 67 (1940).

(124) Fildes, P.: Brit. J. Exptl. Path. **22**, 293 (1941).

(125) Findlay, G. M.: *Recent Advances in Chemotherapy*. P. Blakiston's Son, Philadelphia (1939).

(126) Findlay, G. M.: Brit. J. Exptl. Path. **21**, 356 (1940).

(127) Fitzgerald, R. J., and Babbitt, D.: J. Immunol., in press.

(128) Fitzgerald, R. J., and Lee, M. E.: J. Immunol., in press.

(129) Foster, J. W.: J. Bact. **48**, 97 (1944).

(130) Fox, C. L., Jr., and Rose, H. M.: Proc. Soc. Exptl. Biol. Med. **50**, 142 (1942).
(131) Franklin, A. L., and Chaikoff, I. L.: J. Biol. Chem. **148**, 719 (1943).
(132) Franklin, A. L., and Chaikoff, I. L.: J. Biol. Chem. **152**, 295 (1944).
(133) Franklin, A. L., Chaikoff, I. L., and Lerner, S. R.: J. Biol. Chem. **153**, 151 (1944).
(134) Franklin, A. L., Lerner, S. R., and Chaikoff, I. L.: Endocrinology **34**, 265 (1944).
(135) Gaddum, J. H.: J. Physiol. **61**, 141 (1926).
(136) Gaddum, J. H.: J. Physiol. (Proceedings) **89**, 7 (1937).
(137) Gaddum, J. H.: Trans. Faraday Soc. **39**, 323 (1943).
(138) Gaddum, J. H., and Kwiatkowski, J.: J. Physiol. **94**, 87 (1938).
(139) Geiger, W. B., and Conn, J. E.: J. Am. Chem. Soc. **67**, 112 (1945).
(140) Gilbert, A. J., and Goldman, F.: Proc. Soc. Exptl. Biol. Med. **44**, 458 (1940).
(141) Gladstone, G. P.: Brit. J. Exptl. Path. **20**, 189 (1939).
(142) Glavind, J., and Jansen, K. F.: Acta Physiol. Scand. **8**, 173 (1944); Chem. Abstracts **39**, 4976 (1945).
(143) Glock, G. E.: Nature **154**, 460 (1944).
(144) Goetchius, G. R., and Lawrence, C. A.: J. Bact. **49**, 575 (1945).
(145) Gordon, W. G., and Jackson, R. W.: J. Biol. Chem. **110**, 151 (1935).
(146) Gould, R. G., Kane, L. W., and Mueller, J. H.: J. Bact. **47**, 287 (1944).
(147) Gredner, K., and Schumrick, W.: Arch. exptl. Path. Pharmakol. **202**, 155 (1943) (from reference 283).
(148) Green, H. N.: Brit J. Exptl. Path. **21**, 38 (1940).
(149) Green, H. N., and Bielschowski, F.: Brit. J. Exptl. Path. **23**, 13 (1942).
(150) Gross, R. E.: Z. physiol. Chem. **112**, 236 (1921).
(151) Guggenheim, M.: *Die biogenen Amine.* S. Karger, Basel (1940).
(152) György, P., Melville, D. B., Burk, D., and du Vigneaud, V.: Science **91**, 243 (1940).
(153) Haldane, J. B. S.: *Enzymes.* Longmans, Green and Company, New York (1930).
(154) Hallenbeck, G. A.: Am. J. Physiol. **139**, 329 (1943).
(155) Halpern, B. N.: Compt. rend. soc. biol. **131**, 509 (1939).
(156) Halpern, B. N.: Arch. intern. pharmacodynamie **68**, 339 (1942).
(157) Harrington, C. R.: J. Chem. Soc. **1944**, 193.
(158) Harrington, C. R.: Proc. Roy. Soc. (London) **132**, 223 (1944).
(159) Harris, J. S., and Kohn, H. I.: J. Pharmacol. **73**, 383 (1941).
(160) Harris, J. S., and Kohn, H. I.: J. Biol. Chem. **141**, 989 (1941).
(161) Harris, S. A., Wolf, D. E., Mozingo, R., and Folkers, K.: Science **97**, 447 (1943).
(162) Harris, S. A., Wolf, D. E., Mozingo, R., Anderson, R. C., Arth, G. E., Heyl, D., Wilson, A. N., and Folkers, K.: J. Am. Chem. Soc. **66**, 1756 (1944).
(163) Hartelius, V.: Naturwissenschaften **31**, 440 (1943).
(164) Hartridge, H., and Roughton, F. J. W.: Proc. Roy. Soc. (London) **B94**, 336 (1923).
(165) Harvey, A. M.: Bull. Johns Hopkins Hosp. **65**, 223 (1939).
(166) Hawking, F.: J. Pharmacol. **59**, 123 (1937).
(167) Hawking, F.: Brit. J. Exptl. Path. **25**, 63 (1944).
(167a) Hazard, R., Corteggiani, E., and Pelou, A.: Compt. rend. soc. biol. **138**, 427 (1944).
(168) Hellerman, L.: Physiol. Rev. **17**, 454 (1937).
(169) Hellerman, L.: Cold Spring Harbor Symposia Quant. Biol. **7**, 165 (1939).
(170) Henry, R. J.: Bact. Rev. **7**, 175 (1943).
(171) Henry, R. J.: *The Mode of Action of Sulfonamides,* Publication of the Josiah Macy Jr. Foundation, Review Series, **2**, No. 1 (1944).
(172) Higgins, G. M.: Am. J. Med. Sci. **210**, 347 (1945).
(173) Hill, J. H., and Martin, L.: Medicine **11**, 141 (1932).
(174) Himsworth, H. P.: Lancet **1943**, **II**, 465.

(175) Hirsch, J.: Science **96**, 139 (1942).
(176) Hitchings, G. H., Falco, E. A., and Sherwood, M. B.: Science **102**, 251 (1945).
(177) Hofmann, K.: J. Am. Chem. Soc. **67**, 694 (1945).
(178) Hofmann, K., McCoy, R. H., Felton, J. R., Axelrod, A. E., and Pilgrim, F. J.: Arch. Biochem. **7**, 393 (1945).
(178a) Hofmann, K., and Winnick, T.: J. Biol. Chem. **160**, 449 (1945).
(179) Housewright, R. D., and Koser, S. A.: J. Infectious Diseases **75**, 113 (1944).
(180) Hüfner, G.: J. prakt. Chem. [2] **30**, 68 (1884).
(181) Isaacs, M. L.: Science **75**, 46 (1932).
(182) Isbell, H.: J. Biol. Chem. **144**, 567 (1942).
(183) Ivanovics, G.: Z. Immunitäts. **101**, 58 (1941).
(184) Ivanovics, G.: Z. Immunitäts. **102**, 238 (1942).
(185) Ivanovics, G.: Naturwissenschaften **30**, 104 (1942).
(186) Ivanovics, G.: Z. physiol. Chem. **276**, 33 (1942).
(187) Jadassohn, W., Fierz-David, H. E., and Vollenweider, H.: Schweiz. med. Wochschr. **73**, 122 (1943); Chem. Abstracts **38**, 6362 (1944).
(188) Jadassohn, W., Fierz-David, H. E., and Vollenweider, H.: Helv. Chim. Acta **27**, 1384 (1944); Chem. Abstracts **39**, 3836 (1945).
(189) Jansen, K. F., and Jensen, K. A.: Z. physiol. Chem. **277**, 66 (1942).
(190) Johnson, F. H., and Lewin, I.: Science **101**, 281 (1945).
(191) Johnson, O. H., Green, D. E., and Pauli, R.: J. Biol. Chem. **153**, 37 (1944).
(192) Kabat, H., Stohlman, E. F., and Smith, M. I.: J. Pharmacol. **80**, 160 (1944).
(193) Kaplan, H., and Leubner, G. W.: J. Am. Chem. Soc. **67**, 1076 (1945).
(194) Kennedy, T. H.: Nature **150**, 233 (1942).
(195) Keston, A. S., Goldsmith, E. D., Gordon, A. S., and Charipper, H. A.: J. Biol. Chem. **152**, 241 (1944).
(196) Kimmig, J.: Klin. Wochschr. **20**, 235 (1941).
(197) Klotz, I. M.: J. Am. Chem. Soc. **66**, 459 (1944).
(198) Klotz, I. M., and Gutmann, H. R.: J. Am. Chem. Soc. **67**, 558 (1945).
(199) Knight, B. C. J. G.: Proc. Roy. Soc. Med. **37**, 492 (1944).
(200) Kogl, F., and Borg, W. A. J.: J. physiol. Chem. **281**, 65 (1944); Chem. Abstracts **39**, 4664 (1945).
(201) Kohn, H. I.: Ann. N.Y. Acad. Sci. **44**, 503 (1943).
(202) Kohn, H. I., and Harris, J. S.: J. Pharmacol. **73**, 343 (1941).
(203) Kohn, H. I., and Harris, J. S.: J. Pharmacol **77**, 1 (1943).
(204) Kuhn, R.: Z. Physiol. Chem. **129**, 57 (1923).
(205) Kuhn, R.: Die Chemie **55**, 1 (1942).
(206) Kuhn, R., Möller, E. F., and Wendt, G.: Ber. **76**, 405 (1943).
(207) Kuhn, R., Möller, E. F., Wendt, G., and Beinert, H.: Ber. **75**, 711 (1942).
(208) Kuhn, R., and Schwarz, K.: Ber. **74**, 1617 (1941).
(209) Kuhn, R., Weygand, F., and Möller, E. F.: Ber. **76**, 1044 (1943).
(210) Kuhn, R., Wieland, W., and Möller, E. F.: Ber. **74**, 1605 (1941).
(211) Külz, F.: Arch. exptl. Path. Pharmakol. **98**, 339 (1923).
(212) Kwartler, C., and Lucas, P.: J. Am. Chem. Soc. **65**, 1804 (1943).
(213) Lampen, J. O., and Peterson, W. H.: J. Am. Chem. Soc. **63**, 2283 (1941).
(214) Lampen, J. O., and Peterson, W. H.: Arch. Biochem. **2**, 443 (1943).
(215) Lampen, J. O., Underkofler, L. A., and Peterson, W. H.: J. Biol. Chem. **146**, 277 (1942).
(216) Landau, S. W., and Gay, L. N.: Bull. Johns Hopkins Hosp. **74**, 55 (1944).
(217) Landsteiner, K.: *The Specificity of Serological Reactions*. C. C. Thomas, Baltimore (1936).
(218) Landy, M., and Dicken, D. M.: J. Biol. Chem. **146**, 109 (1943).
(219) Landy, M., Dicken, D. M., Bicking, M. M., and Mitchell, W. R.: Proc. Soc. Exptl. Biol. Med. **49**, 441 (1942).
(220) Landy, M., Larkum, N. W., and Oswald, E. J.: Proc. Soc. Exptl. Biol. Med. **52**, 338 (1943).

(221) Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F.: Science **97,** 265 (1943).
(222) Landy, M., and Wyeno, J.: Proc. Soc. Exptl. Biol. Med. **46,** 59 (1941).
(223) Lawrence, C. A., and Goetchius, G. R.: Proc. Soc. Exptl. Biol. Med. **58,** 356 (1945).
(224) Lehmann, G., and Knoefel, P. K.: J. Pharmacol. **74,** 274 (1942).
(225) Lehmann, G., and Knoefel, P. K.: J. Pharmacol. **80,** 335 (1944).
(226) Lehmann, G., and Young, J. W.: J. Pharmacol. **83,** 90 (1945).
(227) Lehmann, J.: Lancet **1943, I,** 458.
(228) Lehmann, J.: Lancet **1943, I,** 661.
(229) Lester, D.: J. Biol. Chem. **154,** 305 (1944).
(230) Levaditi, C.: Compt. rend. soc. biol. **135,** 1109 (1941).
(231) Levaditi, C., and Pérault, R.: Compt. rend. soc. biol. **135,** 1043 (1941).
(232) Lewis, J. C.: J. Biol. Chem. **146,** 441 (1942).
(233) Lilly, V. G., and Leonian, L. H.: Science **99,** 205 (1944).
(234) Link, K. P.: *Harvey Lectures.* Science Press Printing Company, Lancaster, Pennsylvania (1944).
(235) Link, K. P., Overman, R. S., Sullivan, W. R., Huebner, C. F., and Scheel, L. D.: J. Biol. Chem. **147,** 463 (1943).
(236) Linneweh, F.: Monatsschr. Kinderheilk. **85,** 215 (1940–41) (from reference 216).
(237) Lockwood, J. S.: J. Immunol. **35,** 155 (1938).
(238) Loew, E. R., and Chickering, O.: Proc. Soc. Exptl. Biol. Med. **48,** 65 (1941).
(239) Loew, E. R., and Kaiser, M. E.: Proc. Soc. Exptl. Biol. Med. **58,** 235 (1945).
(240) Loew, E. R., Kaiser, M. E., and Anderson, M.: From reference 75.
(241) Loew, E. R., Kaiser, M. E., and Moore, V.: J. Pharmacol. **83,** 120 (1945).
(242) Loewi, O., and Navratil, E.: Arch. ges. Physiol. (Pflüger's) **214,** 678 (1926).
(243) Loring, H. S.: Ann. Rev. Biochem. **13,** 309 (1944).
(244) Lucas, S. P., and Aggeler, P. M.: Proc. Soc. Exptl. Biol. Med. **56,** 36 (1944).
(245) Lwoff, A., Nitti, F., Tréfouël, Mme. J., and Hamon, V.: Ann. inst. Pasteur **67,** 9 (1941).
(246) McCarty, M.: Proc. Soc. Exptl. Biol. Med. **46,** 133 (1941).
(247) McGinty, D. A.: From reference 16.
(248) McGinty, D. A., and Bywater, W. G.: J. Pharmacol. **84,** 342 (1945).
(248a) McGinty, D. A., and Bywater, W. G.: J. Pharmacol. **85,** 129 (1945).
(249) McIlwain, H.: Brit. J. Exptl. Path. **21,** 136 (1940).
(250) McIlwain, H. Nature **146,** 653 (1940).
(251) McIlwain, H. J. Chem. Soc. **1941,** 75.
(252) McIlwain, H. Brit. J. Exptl. Path. **22,** 148 (1941).
(253) McIlwain, H. Biochem. J. **35,** 1311 (1941).
(254) McIlwain, H. Brit. J. Exptl. Path. **23,** 95 (1942).
(255) McIlwain, H. Biochem. J., **36,** 417 (1942).
(256) McIlwain, H. Lancet **1942, I,** 412.
(257) McIlwain, H. Nature **151,** 270 (1943).
(258) McIlwain, H. Biochem. J. **37,** 265 (1943).
(259) McIlwain, H. Brit. J. Exptl. Path. **24,** 203, 212 (1943).
(260) McIlwain, H. Biochem. J. **38,** 97 (1944).
(261) McIlwain, H. Biol. Rev. Cambridge Phil. Soc. **19,** 135 (1944).
(262) McIlwain, H. Biochem. J. (Proceedings) **38,** 28 (1944).
(263) McIlwain, H.: Biochem. J. **39,** 279 (1945).
(263a) McIlwain, H.: Biochem. J. **39,** 329 (1945).
(264) McIlwain, H., and Hawking, F.: Lancet **1943, I,** 459.
(265) McIlwain, H., and Hughes, D. E.: Biochem. J. **38,** 187 (1944).
(266) McIlwain, H., and Hughes, D. E.: Biochem. J. **39,** 133 (1945).

(267) McIntosh, J., and Whitby, L. E. H.: Lancet **1939, I,** 431.

(268) Mackay, M.: Australian J. Exptl. Biol. Med. Sci. **16,** 137 (1938).

(269) Mackenzie, J. B., and Mackenzie, C. G.: Federation Proc. **1,** 122 (1942).

(270) Mackenzie, C. G., and Mackenzie, J. B.: Endocrinology **32,** 185 (1943).

(271) Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V.: Science **94,** 518 (1941).

(272) MacLeod, C. M.: J. Exptl. Med. **41,** 215 (1940).

(273) Madinaveitia, J.: Biochem. J. (Proceedings) **38,** 27 (1944).

(274) Madinaveitia, J., Martin, A. R., Rose, F. L., and Swain, G.: Biochem. J. **39,** 85 (1945).

(275) Maier, J., and Riley, E.: Proc. Soc. Exptl. Biol. Med. **50,** 152 (1942).

(276) Marrack, J. R.: *The Chemistry of Antigens and Antibodies.* His Majesty's Stationery Office, London (1939).

(277) Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J.: J. Pharmacol. **75,** 89 (1942).

(278) Martin, A. R., and Rose, F. L.: Biochem. J. **39,** 91 (1945).

(279) Martin, A. R., Rose, F. L., and Swain, G.: Nature **154,** 639 (1944).

(280) Martin, G. J., and Fisher, C. V.: J. Biol. Chem. **144,** 289 (1942).

(281) Martin, G. J., and Fisher, C. V.: J. Lab. Clin. Med. **29,** 383 (1944).

(282) Matti, J., Nitti, F., Morel, M., and Lwoff, A.: Ann. inst. Pasteur **67,** 240 (1941).

(283) Mayer, R. L.: To be published.

(284) Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Federation Proc. **4,** 129 (1945).

(285) Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Science **102,** 93 (1945).

(286) Melville, D. B., Dittmer, K., Brown, G. B., and du Vigneaud, V.: Science **98,** 497 (1943).

(287) Mendez, R.: J. Pharmacol. **32,** 451 (1928).

(288) Mentzer, C., and Meunier, P.: Bull. soc. chim. biol. **25,** 379 (1943).

(289) Meunier, P., and Mentzer, C.: Bull. soc. chim. biol. **25,** 80 (1943).

(290) Meunier, P., Mentzer, C., Buu-Hoï, and Cagniant: Bull. soc. chim. biol. **25,** 384 (1943).

(291) Miller, A. K., and Peters, L.: Arch. Biochem. **6,** 281 (1944).

(292) Miller, J. K.: J. Pharmacol. **71,** 14 (1941).

(293) Miller, J. K.: J. Pharmacol., **72,** 354 (1941).

(294) Miller, W. H., Anderson, G. W., Madison, R. K., and Salley, D. J.: Science **100,** 340 (1944).

(295) Miller, W. H., Roblin, R. O., Jr., and Astwood, E. B.: J. Am. Chem. Soc. **67,** 2201 (1945).

(296) Mitchell, H. H.: In *Vitamins and Hormones.* Academic Press, Inc., New York (1943).

(297) Modern, F. S., and Thienes, C. S.: Arch. intern. pharmacodynamie **53,** 413 (1936).

(298) Möller, E. F., and Birkofer, L.: Ber. **75,** 1108, 1118 (1942).

(299) Möller, E. F., and Schwarz, K.: Ber. **74,** 1612 (1941).

(300) Morris, H. C., and Dragstedt, C. A.: Proc. Soc. Exptl. Biol. Med. **59,** 311 (1945).

(301) Morrison, R. S., and Lissak, K.: Am. J. Physiol. **123,** 404 (1938).

(302) Muir, R. D., Shamleffer, V., and Jones, L. R.: J. Bact. (Proceedings) **41,** 84 (1941).

(303) Muir, R. D., Shamleffer, V., and Jones, L. R.: J. Bact. **44,** 95 (1942).

(304) Murray, D. R. P.: Biochem. J. **23,** 292 (1929).

(305) Myrbäck, K.: Z. physiol. Chem. **158,** 160 (1926).

(306) Nielsen, N.: Naturwissenschaften **31,** 146 (1943).

(307) Nielsen, N., and Hartelius, V.: Compt. rend. trav. lab. Carlsberg **22,** 271 (1938).

(308) Nielsen, N., Hartelius, V., and Johansen, G.: Naturwissenschaften **32,** 294 (1944).

(309) Nielsen, N., and Johansen, G.: Naturwissenschaften **31,** 235 (1943).

(310) NORTHEY, E. H.: Chem. Rev. **27**, 85 (1940).

(311) NORTHEY, E. H.: *Sulfonamides and Allied Compounds*. Reinhold Publishing Corporation, New York (in press).

(312) NUNGESTER, W. J., HOOD, M. N., AND WARREN, M. K.: Proc. Soc. Exptl. Biol. Med. **52**, 287 (1943).

(313) OVERMAN, R. S., STAHMANN, M. A., HUEBNER, C. F., SULLIVAN, W. R., SPERO, L., DOHERTY, D. G., IKAWA, M., GRAF, L., ROSEMAN, S., AND LINK, K. P.: J. Biol. Chem. **153**, 5 (1944).

(314) OVERMAN, R. S., STAHMANN, M. A., AND LINK, K. P.: J. Biol. Chem. **145**, 155 (1942).

(315) OVERMAN, R. S., STAHMANN, M. A., SULLIVAN, W. R., HUEBNER, C. F., CAMPBELL, H. A., AND LINK, K. P.: J. Biol. Chem. **142**, 941 (1942).

(316) PARK, C. R., AND WOOD, W. B., JR.: Bull. Johns Hopkins Hosp. **70**, 19 (1942).

(317) PAULING, L., AND PRESSMAN, D.: J. Am. Chem. Soc. **67**, 1003 (1945).

(318) PÉRAULT, R., AND GREIB, E.: Compt. rend. soc. biol. **138**, 506 (1944).

(319) PETERS, L.: J. Pharmacol. **79**, 32 (1943).

(320) PETERSON, C. G., AND PETERSON, D. R.: J. Pharmacol. **84**, 236 (1945).

(321) PETERSON, W. H.: Biol. Symposia **5**, 31 (1941).

(322) PETROVA, A. N.: Advances in Modern Biology (U.S.S.R.) **15**, 65 (1942).

(323) PFALTZ, H.: Z. Vitaminforsch. **13**, 236 (1943); Chem. Abstracts **38**, 6339 (1944).

(323a) PILGRIM, F. J., AXELROD, A. E., WINNICK, T., AND HOFMANN, K.: Science **102**, 35 (1945).

(324) POLLACK, M. A.: J. Am. Chem. Soc. **65**, 1335 (1943).

(325) PORTER, J. R., AND MEYERS, F. P.: Arch. Biochem. **8**, 169 (1945).

(326) QUASTEL, J. H.: Physiol. Rev. **19**, 135 (1939).

(327) QUASTEL, J. H.: Trans. Faraday Soc. **39**, 348 (1943).

(328) QUASTEL, J. H., AND WOOLDRIDGE, W. R.: Biochem. J. **22**, 689 (1928).

(329) QUICK, A. J.: Physiol. Rev. **24**, 297 (1944).

(329a) QUICK, A. J.: J. Biol. Chem. **161**, 33 (1945).

(330) RAIZISS, G. W., SEVERAC, M., AND MOETSCH, J. C.: J. Am. Pharm. Assoc. (Sci. Ed.) **31**, 198 (1942).

(331) RAMANAMANJARY, W.: Compt. rend. soc. biol. **138**, 480 (1944).

(332) RAPOPORT, S., WING, M., AND GUEST, M. G.: Proc. Soc. Exptl. Biol. Med. **53**, 40 (1943).

(333) RAVENTÓS, J.: Quart. J. Exptl. Physiol. **26**, 361 (1937).

(334) RAWSON, R. W., TANNHEIMER, J. F., AND PEACOCK, W.: Endocrinology **34**, 245 (1944).

(335) REINER, L., AND LEONARD, C. S.: Arch. intern. pharmacodynamie **43**, 49 (1932).

(336) RENNICK, B., CHESS, D., HAYS, H. W., MATHIESON, D., MAYER, R. L., AND YONKMAN, F. F.: Federation Proc. **4**, 133 (1945).

(336a) RICHARDS, R. K., EVERETT, G. M., AND KUETER, K. E.: J. Pharmacol. **84**, 387 (1945).

(337) RICHTER, C. P., AND CLISBY, K. H.: Arch. Path. **33**, 46 (1942).

(338) RIEVESCHL, G., JR., AND HUBER, W. F.: Unpublished results.

(339) ROBBINS, W. J.: Proc. Natl. Acad. Sci. U.S. **27**, 419 (1941).

(340) ROBLIN, R. O., JR., AND BELL, P. H.: Ann. N.Y. Acad. Sci. **44**, 449 (1943).

(341) ROBLIN, R. O., JR., LAMPEN, J. O., ENGLISH, J. P., COLE, Q. P., AND VAUGHAN, J. R., JR.: J. Am. Chem. Soc. **67**, 290 (1945).

(342) ROBLIN, R. O., JR., WILLIAMS, J. H., WINNEK, P. S., AND ENGLISH, J. P.: J. Am. Chem. Soc. **62**, 2002 (1940).

(343) ROBLIN, R. O., JR., AND WINNEK, P. S.: J. Am. Chem. Soc. **62**, 1999 (1940).

(344) ROBLIN, R. O., JR., WINNEK, P. S., AND ENGLISH, J. P.: J. Am. Chem. Soc. **64**, 567 (1942).

(345) ROCHA E SILVA, M.: J. Pharmacol. **77**, 198 (1943).

(346) ROCHA E SILVA, M.: J. Pharmacol. **80**, 399 (1944).

(347) ROEPKE, R. R., AND JONES, M. J.: Unpublished results.

(348) Roepke, R. R., Perkins, A. B., and Mercer, F.: To be published.
(349) Rona, P., and Pavlovic, R.: Biochem. Z. **130**, 225 (1922).
(350) Rose, H. M., and Fox, C. L., Jr.: Science **95**, 412 (1943).
(351) Rosenberg, H. R.: *Chemistry and Physiology of the Vitamins.* Interscience Publishers, New York (1942).
(352) Rosenthal, S. M., and Voegtlin, C.: J. Pharmacol. **39**, 347 (1930).
(353) Rosenthal, S. R., and Brown, M. L.: J. Immunol. **38**, 259 (1940).
(354) Rosenthal, S. R., and Minard, D.: J. Exptl. Med. **70**, 415 (1939).
(355) Rubbo, S. D., and Gillespie, J. M.: Nature **146**, 838 (1940).
(356) Rubbo, S. D., Maxwell, M., Fairbridge, R. L., and Gillespie, J. M.: Australian J. Exptl. Biol. Med. Sci. **19**, 185 (1941).
(357) Rubin, S. H., Drekter, L., and Moyer, E. H.: Proc. Soc. Exptl. Biol. Med. **58**, 352 (1945).
(358) Rubin, S. H., Flower, D., Rosen, F., and Drekter, L.: Arch. Biochem. **8**, 79 (1945).
(359) Sarett, H. P.: Federation Proc. **4**, 101 (1945).
(360) Sarett, H. P., and Cheldelin, V. H.: J. Biol. Chem. **156**, 91 (1944).
(361) Sarett, H. P., and Cheldelin, V. H.: J. Bact. **49**, 31 (1945).
(362) Schaumann, O.: Arch. exptl. Path. Pharmakol. **138**, 208 (1928).
(363) Schmelkes, F. C., and Rubin, M.: J. Am. Chem. Soc. **66**, 1631 (1944).
(364) Schmelkes, F. C., Wyss, O., Marks, H. C., Ludwig, B. J., and Strandskov, F. B.: Proc. Soc. Exptl. Biol. Med. **50**, 145 (1942).
(365) Sealock, R. R., and Goodland, R. L.: J. Am. Chem. Soc. **66**, 507 (1944).
(366) Seeler, A. O.: Proc. Soc. Exptl. Biol. Med. **57**, 113 (1944).
(367) Seeler, A. O., Graessle, O., and Dusenbery, E. D.: J. Bact. **45**, 205 (1943).
(368) Selbie, F. R.: Brit. J. Exptl. Path. **21**, 90 (1940).
(369) Sevag, M. G., Henry, J., and Richardson, R. A.: J. Bact. **49**, 71 (1945).
(370) Sevag, M. G., Richardson, R. A., and Henry, J.: J. Bact. **49**, 79 (1945).
(371) Sevag, M. G., and Shelburne, M.: J. Bact. **43**, 447 (1942).
(372) Sevag, M. G., Shelburne, M., and Mudd, S.: J. Gen. Physiol. **25**, 805 (1942).
(373) Sevag, M. G., Shelburne, M., and Mudd, S.: J. Bact. **49**, 65 (1945).
(374) Shapiro, S., Redish, M. H., and Campbell, H. A.: Proc. Soc. Exptl. Biol. Med. **52**, 12 (1943).
(375) Shive, W., and Snell, E. E.: Science **102**, 401 (1945).
(375a) Shive, W., and Snell, E. E.: J. Biol. Chem. **160**, 287 (1945).
(376) Silvermann, M., and Evans, E. A., Jr.: J. Biol. Chem. **150**, 265 (1943).
(377) Silvermann, M., and Evans, E. A., Jr.: J. Biol. Chem. **154**, 521 (1944).
(378) Simon, C. E., and Wood, M. A.: Am. J. Med. Sci. **147**, 524 (1914).
(379) Singer, T. P., and Barron, E. S. G.: J. Biol. Chem. **157**, 241 (1945).
(380) Snell, E. E.: J. Biol. Chem. **139**, 975 (1941); **141**, 121 (1941).
(381) Snell, E. E.: Arch. Biochem. **2**, 389 (1943).
(382) Snell, E. E.: J. Biol. Chem. **152**, 475 (1944).
(383) Snell, E. E., Chan, L., Spiridanoff, S., Way, E. L., and Leake, C. D.: Science **97**, 168 (1943).
(384) Snell, E. E., and Guirard, B. M.: Proc. Nat. Acad. Sci. **29**, 66 (1943).
(385) Snell, E. E., and Mitchell, H. K.: Arch. Biochem. **1**, 93 (1942).
(386) Snell, E. E., and Shive, W.: J. Biol. Chem. **158**, 551 (1945).
(387) Sobotka, H., and Glick, D.: J. Biol. Chem. **105**, 199 (1934).
(388) Soodak, M., and Cerecedo, L. R.: J. Am. Chem. Soc. **66**, 1988 (1944).
(389) Spink, W. W., and Jermsta, J.: Proc. Soc. Exptl. Biol. Med. **47**, 395 (1941).
(390) Spink, W. W., Vivino, J. J., and Mickelson, O.: Proc. Soc. Exptl. Biol. Med. **50**, 31 (1942).
(391) Spizizen, J.: J. Infectious Diseases **73**, 212 (1943).
(392) Stahmann, M. A., Huebner, C. F., and Link, K. P.: J. Biol. Chem. **138**, 513 (1941).

(393) Stamp, T. C.: Lancet **1939, II,** 10.

(394) Stansly, P. G., and Alverson, C. M.: Science, in press.

(395) Stansly, P. G., and Schlosser, M. E.: J. Biol. Chem. **161,** 513 (1945).

(396) Staub, A.-M.: Ann. inst. Pasteur **63,** 400 (1939).

(397) Staub, A.-M.: Ann. inst. Pasteur **63,** 485 (1939).

(398) Staub, A.-M., and Bovet, D.: Compt. rend. soc. biol. **125,** 818 (1937).

(399) Stedman, E., and Stedman, E.: Biochem. J. **25,** 1147 (1931).

(400) Stephenson, M.: *Bacterial Metabolism.* Longmans, Green and Company, London (1939).

(401) Stearn, E. W., and Stearn, A. E.: J. Bact. **9,** 491 (1924).

(402) Stokes, J. L.: J. Bact. **48,** 201 (1944).

(403) Strangeways, W. I.: Ann. Trop. Med. Parasitol. **31,** 387 (1937).

(404) Straus, E., Dingle, J. H., and Finland, M.: J. Immunol. **42,** 313, 331 (1941).

(405) Strauss, E., Lowell, F. C., and Finland, M.: J. Clin. Investigation **20,** 189 (1941).

(406) Sumner, J. B., and Somers, G. F.: *Enzymes.* Academic Press, New York (1943).

(407) Tammann, G.: Z. physiol. Chem. **16,** 271 (1891).

(408) Tamura, J. T.: J. Bact. **47,** 529 (1944).

(408a) Tatum, E. L.: J. Biol. Chem. **160,** 455 (1945).

(409) Tatum, E. L., and Beadle, G. W.: Proc. Natl. Acad. Sci. U. S. **28,** 234 (1942).

(410) Teague, P. C., and Williams, R. J.: J. Gen. Physiol. **25,** 777 (1942).

(411) Tepley, L. J., Axelrod, A. E., and Elvehjem, C. A.: J. Pharmacol. **77,** 207 (1943).

(412) Thimann, K. V.: Arch. Biochem. **2,** 87 (1943).

(413) Tracy, A. H., and Elderfield, R. C.: J. Org. Chem. **6,** 54 (1941).

(414) Ungar, G., Parrot, J.-L., and Bovet, D.: Compt. rend. soc. biol. **124,** 445 (1937).

(415) Unna, K.: Proc. Soc. Exptl. Biol. Med. **54,** 55 (1943).

(416) Valko, E. I., and DuBois, A. S.: J. Bact. **47,** 15 (1944).

(417) Vallery-Radot, P., Bovet, D., Mauric, G., and Holtzer, A.: Compt. rend. soc. biol. **136,** 354 (1942).

(418) Vallery-Radot, P., Bovet, D., Mauric, G., and Holtzer, A.: Compt. rend. soc. biol. **136,** 385 (1942).

(419) Vigneaud, V. du: Science **96,** 455 (1942).

(420) Vigneaud, V. du, McKennis, H., Jr., Simmonds, S., Dittmer, K., and Brown, G. B.: J. Biol. Chem. **159,** 385 (1945).

(421) Vigneaud, V. du, Melville, D. B., Folkers, K., Wolf, D. E., Mozingo, R., Keresztesy, J. C., and Harris, S. A.: J. Biol. Chem. **146,** 475 (1942).

(422) Voegtlin, C.: Physiol. Rev. **5,** 63 (1925).

(423) Voegtlin, C., Dyer, H. A., and Leonard, C. S.: U.S. Pub. Health Rep. **38,** 1882 (1923).

(424) Voegtlin, C., Johnson, J. M., and Dyer, H. A.: Proc. Natl. Acad. Sci. U.S. **11,** 344 (1925).

(425) Wagner-Jauregg, Th.: Naturwissenschaften **31,** 335 (1943).

(426) Weinstock, H. H., Jr., May, E. L., Arnold, A., and Price, D.: J. Biol. Chem. **135,** 343 (1940).

(427) Weis, O., and Jones, L. R.: J. Bact. (Proceedings) **41,** 82 (1941).

(428) Welch, A. D.: Physiol. Rev. **25,** 687 (1945).

(429) Wells, J. A., and Morris, H. C.: Federation Proc. **4,** 140 (1945).

(429a) Wells, J. A., Morris, H. C., Bull, H. B., and Dragstedt, C. A.: J. Pharmacol. **85,** 122 (1945).

(430) West, R., and Coburn, A. F.: J. Exptl. Med. **72,** 91 (1940).

(431) Wiedling, S.: Science **94,** 389 (1941).

(432) White, H. J.: Unpublished results.

(433) White, H. J., Lee, M., Jackson, E. R., Himes, A., and Alverson, C. M.: To be published.

(434) Wilcox, H. B., Jr., and Seegal, B. C.: J. Immunol. **44,** 219 (1942).
(435) Williams, R. H., and Bissell, G. W.: Science **98,** 156 (1943).
(435a) Williams, R. H., and Frame, E. G.: Bull. Johns Hopkins Hosp. **77,** 314 (1945).
(436) Williams, R. J.: In *Advances in Enzymology.* Interscience Publishers, New York (1943).
(437) Winterbottom, R., Clapp, J. W., Miller, W. H., English, J. P., and Roblin, R. O., Jr.: To be published.
(438) Witts, L. J.: Glasgow Med. J. **19,** 57 (1942).
(439) Wohl, A., and Glimm, E.: Biochem. Z. **27,** 349 (1910).
(440) Wood, J. L. and du Vigneaud, V.: J. Am. Chem. Soc. **67,** 210 (1945).
(441) Wood, W. B., Jr.: J. Exptl. Med. **75,** 369 (1942).
(442) Wood, W. B., Jr., and Austrian, R.: J. Exptl. Med. **75,** 383 (1942).
(443) Woods, D. D.: Brit. J. Exptl. Path. **21,** 74 (1940).
(444) Woods, D. D.: Biochem. J. **36,** 4 (1942).
(445) Woods, D. D.: Personal communication.
(446) Woods, D. D., and Fildes, P.: J. Soc. Chem. Ind. **59,** 133 (1940).
(447) Woolley, D. W.: J. Biol. Chem. **152,** 225 (1944).
(448) Woolley, D. W.: J. Biol. Chem. **154,** 31 (1944).
(449) Woolley, D. W.: Proc. Soc. Exptl. Biol. Med. **55,** 179 (1944).
(450) Woolley, D. W.: Federation Proc. **3,** 97 (1944).
(451) Woolley, D. W.: Science **100,** 579 (1944).
(452) Woolley, D. W.: J. Biol. Chem. **157,** 455 (1945).
(453) Woolley, D. W.: J. Biol. Chem. **159,** 59 (1945).
(453a) Woolley, D. W.: Proc. Soc. Exptl. Biol. Med. **60,** 225 (1945).
(454) Woolley, D. W., and Collyer, M. L.: J. Biol. Chem. **159,** 263 (1945).
(455) Woolley, D. W., and Krampitz, L. O.: J. Exptl. Med. **78,** 333 (1943).
(456) Woolley, D. W., Strong, F. M., Elvehjem, C. A., and Madden, R. J.: J. Biol. Chem. **124,** 715 (1938).
(457) Woolley, D. W., and White, A. G. C.: J. Biol. Chem. **149,** 285 (1943).
(458) Woolley, D. W., and White, A. G. C.: J. Exptl. Med. **78,** 489 (1943).
(459) Woolley, D. W., and White, A. G. C.: Proc. Soc. Exptl. Biol. Med. **52,** 106 (1943).
(460) Wright, H. N., and Hirschfelder, A. D.: J. Pharmacol. **39,** 39 (1930).
(461) Wyss, O.: Proc. Soc. Exptl. Biol. Med. **48,** 122 (1941).
(462) Wyss, O.: J. Bact. **46,** 483 (1943).
(463) Wyss, O., Grubaugh, K. K., and Schmelkes, F. C.: Proc. Soc. Exptl. Biol. Med. **49,** 618 (1942).
(464) Wyss, O., Rubin, M., and Strandskov, F. B.: Proc. Soc. Exptl. Biol. Med. **52,** 155 (1943).
(465) Yonkman, F. F., Chess, D., Mathieson, D., and Hansen, N.: Federation Proc. **4,** 143 (1945).
(466) Yonkman, F. F., Hays, H. W., and Rennick, B.: Federation Proc. **4,** 144 (1945).
(467) Yorke, W., Murgatroyd, F., and Hawking, F.: Ann. Trop. Med. Parasitol. **25,** 351 (1931).
(468) Young, R. H., and Gilbert, R. P.: J. Allergy **12,** 235 (1941).
(469) Yuan, H. C., and Li, H. C.: J. Chinese Chem. Soc. **5,** 214 (1937) (from reference 420).
(470) Zeller, E. A.: Helv. Chim. Acta **21,** 1645 (1938).
(471) Zentmyer, G. A.: Science **100,** 294 (1944).

FERRITIN: ITS PROPERTIES AND SIGNIFICANCE FOR IRON METABOLISM

S. GRANICK

The Laboratories of The Rockefeller Institute for Medical Research, New York City, New York

Received September 19, 1945

CONTENTS

I. Introduction. Kinds of storage iron 380
II. History of hemosiderin and ferritin iron 381
III. Hemosiderin 382
IV. Ferritin and diffuse tissue iron 383
  A. Isolation and preparation of crystalline ferritin 383
  B. Preparation of crystalline apoferritin 384
  C. Physical properties of ferritin and apoferritin 384
    1. Ultracentrifuge data 384
    2. Crystallization and crystal structure 385
    3. Effect of heat 386
    4. Absorption spectrum 388
    5. Magnetic susceptibility of ferritin iron 388
  D. Chemical properties 391
    1. Analysis of ferritin 391
    2. Analysis of apoferritin 391
    3. Composition of ferritin-iron micelle 392
  E. Distribution of ferritin 393
    1. Ferritin in species and organs 393
    2. Antigenic studies and cross reactions 394
  F. Microchemical tests for iron in tissues 394
    1. Methods 394
    2. Iron in horse spleen 395
    3. Consideration of iron in nucleoprotein 395
  G. Possible function of ferritin as a mucosal regulator of iron absorption 396
  H. Iron transport in the blood stream 398
  I. Function of ferritin as an iron-storage compound 398
    1. Ferritin in anemic and in normal horses 399
    2. Conversion of tagged hemoglobin iron to ferritin iron 399
    3. Conversion of tagged inorganic iron to ferritin iron 400
    4. Rate of conversion to ferritin iron 400
  J. Some factors in ferritin formation 401
  K. Summary of iron metabolism in relation to ferritin 401
V. References 402

Ferritin is a protein, crystallizable by cadmium sulfate, which may contain as much as 23 per cent by weight of iron. It has been isolated from bone marrow, spleen, and liver of a number of animals and has recently been detected in the gastrointestinal mucosa. The iron can be removed from ferritin without denaturing the protein. The protein thus produced is designated as apoferritin. It is homogeneous, has a molecular weight of 460,000, and crystallizes with cadmium sulfate, the space lattices of the ferritin and the apoferritin crystals being identical. The iron is present as micelles of the approximate composition $[(FeOOH)_8 \cdot (FeOPO_3H_2)]$, firmly bound to the protein. The variable size and concentration of these micelles account for the inhomogeneity of ferritin. The iron is in the ferric

state and is the only biologically occurring iron compound to possess a magnetic moment equivalent to three unpaired electrons per iron atom. Tracer studies with radioactive iron show that inorganic iron and the iron in the hemoglobin of red cells can be rapidly converted in the liver, spleen, and bone marrow into ferritin iron. Ferritin functions as a store of iron readily available for hemin synthesis, the iron being held in small micelles of large surface area on a particular protein, apoferritin, thus protecting the cell from toxic accumulations of ferric hydroxide. It is probable that ferritin may also function in the regulation of iron absorption by the gastrointestinal mucosa.

I. Introduction

With the discovery of a protein iron compound, ferritin, containing 23 per cent of iron, and the advent of radioactive iron as tracer, the study of iron metabolism has entered a new phase of development. It will be the purpose of this review to call attention not only to the few scattered facts recently arrived at but also to the newly recognized and tempting gaps which are so readily filled with conjecture and hypothesis. We shall limit ourselves primarily to a discussion of the properties of ferritin, and of its function as an iron-storage compound and as a possible regulator of iron absorption. Other aspects of iron metabolism will be discussed only as they relate to ferritin.

The total iron in a 70-kg. man averages about 4.3 g. (22). Of this iron, some 55 per cent is contained in the circulating hemoglobin, and about 10 per cent is a constituent of muscle hemoglobin and the heme catalysts. The remainder, 30–35 per cent of the body iron, may be designated as the storage-iron fraction. About two-thirds of this storage iron, or 0.8 g., is located in the liver, spleen, and red bone marrow. As pointed out by Hahn (18), the storage iron in these organs is sufficient, when converted into hemoglobin, to increase the circulating hemoglobin by about 30–50 per cent.

The storage-iron fraction, for purposes of this review, will be divided into three parts: monomolecularly dispersed iron compounds; colloidal iron compounds, namely, ferritin and non-crystallizable ferritin; and a microscopically visible granular iron material, hemosiderin.

The monomolecularly dispersed ferrous and ferric compounds, which are probably in the form of their hydroxides, may be considered to represent the metabolically active iron which may be in equilibrium with the storage-iron compound, ferritin.

The colloidal iron compounds, ferritin and non-crystallizable ferritin, appear to contain iron associated with one or possibly more proteins. The iron is in the form of a reddish brown ferric hydroxide of the approximate composition $[(FeOOH)_8 \cdot (FeOPO_3H_2)]$, the iron being uniquely characterized by magnetic measurements as possessing a constant magnetic dipole moment of 3.8 Bohr magnetons, a moment rarely observed in other iron compounds. The size of the micelles is unknown, but they are probably not uniform in size. Iron micelles with these properties we shall designate as "ferritin–iron micelles", "iron micelles" or "ferritin iron" also being used where no ambiguity arises.

When ferritin–iron micelles are attached to a specific protein, apoferritin (through an unknown chemical linkage), the complex of this protein with the ferritin–iron micelles is designated as ferritin. Apoferritin is a homogeneous protein which may be readily crystallized with cadmium sulfate, yielding colorless crystals. When apoferritin has iron micelles attached, then red-brown crystals of ferritin may be obtained with a maximum amount of iron equivalent to as much as 23 per cent of the dry weight of the dialyzed crystalline material.

Some of the ferritin–iron micelles appear to be attached to proteins which cannot be crystallized. These inhomogeneous materials, which still possess the characteristic ferritin–iron micelles, are placed together into a group termed the "non-crystallizable ferritin" or NCF. Some of this material is undoubtedly denatured ferritin (i.e., denatured apoferritin with attached ferritin–iron micelles). It is possible that there are also apoferritin molecules with relatively large iron micelles which do not crystallize because they cannot fit spacially into the crystal lattice. Possibly still other material may represent two ferritin molecules bound together by cross links between the ferritin–iron micelles of one molecule and the ferritin–iron micelles of the other. It is conceivable that these aggregates might increase to such proportions that they would become microscopically visible and be classed with the hemosiderin granules.

The microscopically visible aggregates of iron pigment have long been familiar to histologists as "hemosiderin" granules. The iron of the granules of the horse spleen can be distinguished from ferritin iron magnetically and also chemically, since some of the granules contain a higher ratio of iron to protein than is present in ferritin.

II. HISTORY OF HEMOSIDERIN AND FERRITIN IRON

In the period 1870–1880 granular deposits in the liver and spleen, which gave the characteristic Prussian blue stain for iron and which are today designated as hemosiderin (38), began to be reported with frequency. These granules varied from colorless through pale yellow to deep brown in color. One of the best materials in which the iron-rich granules are easily visible is the splenic pulp of old horses where, according to Nasse (37), over 5 per cent of the dry weight of the spleen consisted of these granules. Neumann (38) and Perls (43) early recognized these granules to contain an iron oxide and iron phosphate. In other animals, iron-containing granules are not common but become apparent on the rapid breakdown of hemoglobin (55). For example, they are readily seen in the reticular cells of the bone marrow some 36 hr. after the injection of hemoglobin. Besides the reticular tissue of liver, spleen, and bone marrow, there is to a lesser degree a predilection for iron by the globus pallidus and substantia nigra of the central nervous system (48).

Succeeding investigators came to recognize that iron was present not only in granules visible under the microscope, but also in a more diffuse form. According to Schmiedeberg (47), the iron was attached to a protein and this compound, which he called Ferratin, was present in spleen, liver, and marrow. From pig liver, by boiling with water and precipitating with tartaric acid, Schmiedeberg isolated an iron protein containing 6 per cent of iron. (Today we know that this compound was a mixture of denatured ferritin and denatured proteins.) Other investigators, including Zaleski (58), Salkowski (46), and Hammarstein, were of the opinion that the iron extracted and fractionated from liver was closely linked with nucleoproteins. Still another view was held by Burow (5), who claimed the iron to be associated with lipoids in the spleen. Of recent date but of historical vintage is the preparation of denatured ferritin, called ferrin (26).

A beginning in the understanding of the true character of the soluble iron compound was made in a notable contribution by Laufberger (25) in 1937. Laufberger isolated from horse spleen a protein to which he gave the name ferritin, in deference to the work of Schmiedeberg, who had almost isolated this compound. This protein was red-brown in color, contained over 20 per cent by dry weight of iron, and was crystallizable by means of cadmium sulfate. The isolation procedure depended on the protein being stable at 80°C.; thus other proteins could be removed by coagulation, leaving ferritin in solution. Kuhn *et al.* (24) confirmed this work in 1940 and suggested that ferritin was a nucleoprotein with the iron attached at each peptide link. Recent studies, mainly from Michaelis' laboratory, have further characterized this interesting compound. It has been found to be made up of a homogeneous protein, "apoferritin" (of molecular weight 460,000), to which are attached, in chemical linkage, small micelles of iron hydroxide–iron phosphate. The discovery of Michaelis that the iron in these micelles possesses a constant, characteristic magnetic susceptibility which may be interpreted as due to three unpaired electrons per iron atom makes it possible to distinguish ferritin iron from all other biologically occurring iron compounds of the body. Tracer studies in collaboration with Hahn at Whipple's laboratory (21) have shown that ferritin functions as an iron-storage compound of the liver, spleen, and marrow. Recently evidence has accumulated to suggest that ferritin of the gastrointestinal mucosa may play a rôle in the regulation of iron absorption (12, 20).

III. Hemosiderin

A microscopically visible granular iron pigment, hemosiderin, appears generally in the marrow, spleen, and liver after rapid hemoglobin destruction or rapid and excessive iron deposition. Of different origin may be the iron-staining granules found in certain red blood cells (17, 41). The phagocytes of the marrow, spleen, and liver have the property of engulfing damaged red cells and fragments, and decomposing them. The heme is oxidized, releasing the iron and splitting the porphyrin ring into the open-chain biliverdin, which is reduced to bilirubin and finally excreted in the bile. At the same time the globin and stroma are acted upon by proteolytic enzymes, some of the material being denatured and temporarily converted to granules which at first appear yellow-brown and give the iron stain. Within 3 or 4 days the iron stain may disappear, although some of the partially digested protein granules still have a pale yellow tinge, this color being often due to adsorbed bilirubin. (Such granules have been called hematoidin.)

Chemical studies of hemosiderin have been largely confined to granular material obtained from the horse spleen. It is not yet known whether the granules are a result of rapid blood destruction or of an abnormally high absorption of iron or are due to a combination of both of these factors.

Cook (6) and Asher (2) have examined these granules of horse spleen and found them to consist of protein material containing brown iron hydroxide and small amounts of phosphate and calcium. By differential centrifugation in carbon tetrachloride, Asher was able to obtain granules containing as much as 35.9 per cent by weight of iron. These granules, then, may vary in composition from relatively little proteinaceous material and high iron content to relatively low iron content.

Analysis of hemosiderin granules (9) from horse spleen indicated 8.29 per cent iron, 12.9 per cent nitrogen, and 1.6 per cent phosphorus, a composition not too different from that of some ferritin samples. The apparent magnetic moment of the iron in these granules was 3.4 B.U., as contrasted with 3.8 B.U. per gram-atom of ferritin iron (32).

IV. Ferritin and Diffuse Tissue Iron

A. ISOLATION AND PREPARATION OF CRYSTALLINE FERRITIN

Normal horse spleen is the richest source of ferritin. By teasing a piece of horse spleen in 10 per cent cadmium sulfate solution it is possible to observe ferritin crystals forming under the microscope after a few minutes, demonstrating that ferritin is not an artifact of heating, salting out, etc.

The method of isolation (9) is a modification of the one first devised by Laufberger (25) and of that used by Kuhn, Sorensen, and Birkhofer (24). One kilogram of horse spleen is ground in a meat grinder (for smaller amounts of tissue a Waring blendor is useful), mixed with 1.5 liters of water, and heated rapidly in a water bath to 80°C. with stirring. The heavy coagulum of denatured proteins is then removed by first running the hot suspension through coarse cheese cloth, and then the filtrate onto large fluted filters. (Further liquid may be obtained by squeezing the coagulum on the cheese cloth, in a hand press.) A clear brownish solution results. To each 100 cc. of solution is added, with stirring, 35 g. of ammonium sulfate; the resulting suspension is left overnight in the ice box at 0°C. The brown precipitate containing the ferritin is then centrifuged down, and the supernatant liquid sucked off as completely as possible. (For small amounts of precipitate it is advisable to transfer the precipitate as a thick suspension to a dialyzing bag and dialyze away the ammonium sulfate before crystallizing.) The precipitate is now dissolved in a small volume of distilled water, yielding a deep red-brown solution containing ferritin (F) and non-crystallizable ferritin (NCF). For each 100 cc. of this solution is added with stirring 25 cc. of a 20 per cent solution of $CdSO_4 \cdot 8/3H_2O$. Crystallization begins within a few minutes. After 1–2 days at room temperature the crystals are centrifuged down. By differential centrifugation large heavy crystals settle to the bottom and can be separated by sucking off the supernatant mother liquor and a lighter brown sludge. The mother liquor, which is still quite red-brown in color but no longer yields crystals, will be referred to as non-crystallizable ferritin (NCF). Crystalline ferritin is only sparingly soluble in distilled water. It is recrystallized by dissolving it in the smallest possible volume of 2 per cent by weight ammonium sulfate solution, and for each 100 cc. of the clear solution adding 25 cc. of 20 per cent cadmium sulfate solution.

B. PREPARATION OF CRYSTALLINE APOFERRITIN

The removal of the iron micelles from ferritin while maintaining the protein apoferritin undenatured may be brought about (9, 15, 16) by reducing the ferric hydroxide to the ferrous condition in a medium (pH 4.5) which is acid but not acid enough to denature the protein. In a cellophane dialyzing bag of 1 cm. diameter are placed 40 cc. of a 1–2 per cent ferritin solution. This bag is inserted into a long glass tube of 2.5 cm. diameter and 150 cc. capacity, containing acetate buffer pH 4.6, of ionic strength about 1.0. The slight acidity of this buffer is necessary to make possible the reduction of ferric to ferrous iron. Now 2.0 g. of sodium dithionite are added, together with a few glass beads, and the tube is stoppered, the inclusion of air bubbles being avoided. After 24 hr. of intermittent shaking at room temperature the brown solution has turned pale yellow. The cellophane bag is then dialyzed for 24 hr. against running distilled water. The protein solution in the bag is treated with one-fourth of its volume of 20 per cent cadmium sulfate. Pale yellow crystals form almost immediately. After several hours they are centrifuged down, the supernatant liquid is discarded, and the crystals are brought into solution with the smallest volume of 2 per cent ammonium sulfate solution.

In order to remove the last traces of iron from the protein the solution is placed in a cellophane bag, together with 300 mg. of α,α'-bipyridine. The bag is inserted into a glass tube containing the 1 M acetate buffer pH 4.6, 1 g. of sodium dithionite is added plus some glass beads, and the tube is stoppered (avoiding air bubbles) and shaken intermittently for 1 day. The cellophane bag is then removed from the glass tube and is dialyzed for 1 day against running distilled water to remove the ferrous bipyridine. Finally the apoferritin solution is crystallized with one-fourth of its volume of 20 per cent cadmium sulfate. Occasionally the crystals are not quite colorless, but pale yellow, the yellow color being due not to the presence of iron, but rather to traces of cadmium sulfide. The colorless crystals of apoferritin are identical in shape with those of the brown ferritin crystals. It is possible to obtain crystals ranging from deep brown to colorless, with an iron content of from 23 to 0 per cent, without any variation in crystal form. The ability to crystallize as a cadmium salt is evidently a property of apoferritin alone and does not depend upon nor is it interfered with by the combination with the iron micelles, even though these iron micelles can make up 38 per cent by weight of the ferritin crystal.

C. PHYSICAL PROPERTIES OF FERRITIN AND APOFERRITIN

1. Ultracentrifuge data

Careful ultracentrifuge studies of solutions of crystallized ferritin by Rothen (44) have shown that ferritin is inhomogeneous. A colorless protein fraction, apoferritin, makes up 20–25 per cent by weight of the material; the remaining 75–80 per cent is a reddish brown fraction which sediments more rapidly, showing no sharp moving boundary. This latter fraction is made up of small iron hydroxide micelles attached to apoferritin molecules so firmly that they cannot be separated from the protein by differential ultracentrifugation. From the ultracentrifuge data Rothen suggests that the brown material consists of units which on the average contain two apoferritin molecules plus a number of small iron hydroxide micelles. Horse apoferritin has a molecular weight of 465,000; if considered as an ellipsoid, its axis ratio is 3:1. The sedimentation constants of human and horse apoferritin are identical; dog apoferritin has a sedimentation constant about 3 per cent higher. The marked stability and homogeneity of horse apoferritin and its convenient molecular weight have been made use of by Rothen in testing the precision of the ultracentrifuge technique.

2. Crystallization and crystal structure

Ferritin is an inhomogeneous substance, as shown by its variation in nitrogen, phosphorus, and iron content. Its properties of crystallization are due primarily to the protein (apoferritin) which it contains. Apoferritin and ferritin resemble the globulins in several ways. Like apoferritin, the globulins are precipitated in an amorphous state by very low concentrations of the bivalent metal ions, such as zinc, cadmium, nickel, and cobalt. At slightly higher concentrations globulins become soluble again and are not precipitable by further increasing (within certain limits) the concentrations of these ions. In contrast, apoferritin crystallizes readily at higher concentrations, especially with cadmium sulfate and less readily with zinc sulfate. An amorphous precipitate of apoferritin which is formed in a 0.5 per cent cadmium sulfate solution, dissolves in 3 per cent cadmium sulfate and soon deposits large well-formed crystals. On a dry weight basis, the amorphous precipitate is found to contain about 3.5 per cent cadmium; the large crystals contain 6–7 per cent cadmium (9).

The cadmium is considered to be present in two forms. Most of it is to be found in the relatively large pores or capillary spaces that are present in the crystal protein lattice; the remainder is attached to the individual protein molecules. Wet protein crystals appear to be made up of protein molecules with interstices or pore spaces between them that are large and filled with loosely held salt and solvent molecules. If one considers spherical protein molecules as arranged in closest hexagonal packing, the volume of space between the spherical molecules would occupy about 25 per cent of the total volume of the crystal. Often the volume of these interstices in the crystal may approach or even exceed the volume occupied by the protein molecules themselves. For example, from the data of McMeekin and Warner (31) one may calculate that lactoglobulin crystals have an interstitial volume of 53 per cent. In physicochemical studies of proteins it becomes more logical to consider the contents of the interstices as a continuum of the crystallizing mother liquor rather than as "water and salt of crystallization". This interpretation is made readily apparent by the following experiment. When ferritin crystals were suspended in a saturated potassium chloride solution, in which the crystals are insoluble, and spun down rapidly in a centrifuge, the cadmium content of the ferritin crystals was found to decrease from 6.7 per cent to 1.7 per cent. Much of the cadmium sulfate mother liquor which had been present in the interstices of the crystals was evidently swept out by the potassium chloride solution without affecting the crystalline form (9). A portion of the cadmium, however, cannot be displaced; it appears to cling very firmly to the protein and may serve to coördinate the apoferritin molecules into a definite lattice pattern. A comparison, in this respect, with insulin is interesting. Insulin crystallizes as a zinc compound, but an extremely small concentration of zinc is sufficient to bring about crystallization. For the crystallization of ferritin a large excess of cadmium is requisite.

That the interstitial or pore space is of comparatively large magnitude follows also from a comparison of the x-ray studies of apoferritin and ferritin crystals made by Fankuchen (8), so far only with the Debye–Scherrer method, although crystals large enough for the single-crystal method are now available. The data reveal that the distances between the protein units in ferritin and in apoferritin crystals are identical. This is a surprising finding, if one considers that 38 per cent of the original ferritin can be removed as $[(FeOOH)_8 \cdot (FeOPO_3H_2)]$ and the resulting apoferritin will still crystallize with a space lattice identical with that of the original ferritin. The Debye–Scherrer lines for both ferritin and apoferritin are at the same locations but differ in intensity and correspond to a face-centered cubic cell, side 186 Å., having eight molecules per cubic cell. The fact that some of the lines obtained with ferritin are more intense indicates that the iron micelles are spaced at distances identical with the distances from one protein molecule to the next.

The only position that can be postulated satisfying x-ray and other physical and chemical data of the crystals is for the iron micelles to be arranged in the interstices between the protein molecules.

In general, ferritin and apoferritin crystals belong to the cubic system and are isotropic, whatever the species or conditions under which crystallization occurs may be (10) (figure 1). (One exception has been noted. When horse ferritin crystals are suspended in 0.6 per cent cadmium sulfate, small square plates which are optically anisotropic begin to form after several days.) In the mouse, rat, cat, and pig the crystals are octahedra; in the horse they are tetrahedra, octahedra, and most frequently twinned octahedra; in the dog they are tetrahedra; in the guinea pig they are octahedra or rectangular parallelepipeds; in the human they have rounded edges with a suggestion of octahedral form; in the pig the crystals obtained from the testicles are cubic and colorless.

3. Effect of heat

A peculiarity observed with solutions of ferritin which are 2 per cent or higher in concentration, but not with apoferritin, is the effect of heat (9). Dilute ferritin and apoferritin solutions remain clear on heating to 80°C. Above that temperature irreversible coagulation occurs. However, in a more concentrated ferritin solution there appears at 65–70°C. a cloudiness or, according to concentration, a massive coagulation, which disappears spontaneously on cooling. Heating from 70–80°C. delays the rate of clearing at room temperature.

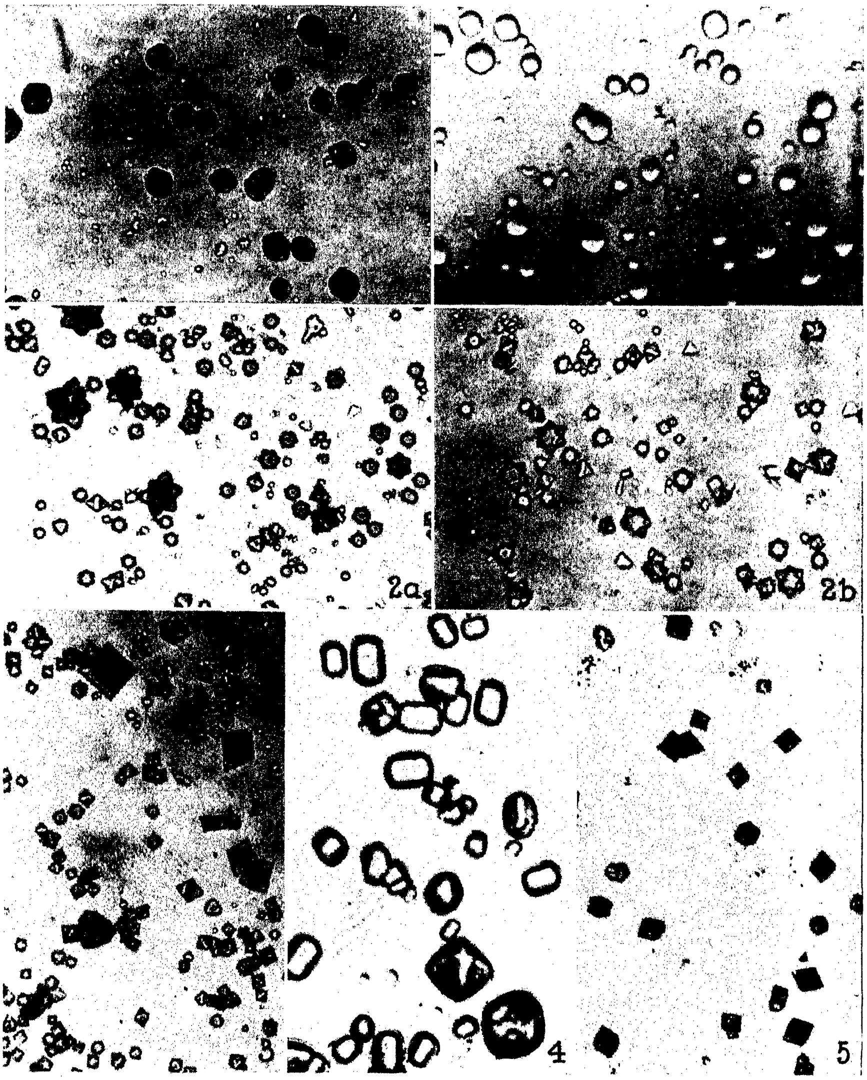


Fig. 1. 1a, human ferritin; 1b, human apoferritin; 2a, horse ferritin; 2b, horse apoferritin; 3, dog ferritin; 4, guinea pig ferritin; 5, mouse ferritin.

In concentrated ferritin solutions at temperatures below 80°C. an aggregation is observed which is spontaneously reversible at lower temperatures. Above 80°C., a denaturation of the protein molecules which is not reversible occurs, such changed molecules agglutinating to insoluble granules.

4. Absorption spectrum

The absorption of apoferritin (figure 2a) is that of a typical protein with a well-defined band at 280 mμ, indicating the presence of aromatic amino acids. The curve of ferritin (figure 2b) is due primarily to the absorption of the red-brown iron micelles and possibly to a certain amount of cadmium sulfide which may be present as an impurity. No absorption band in the region characteristic of nucleic acid has been observed.

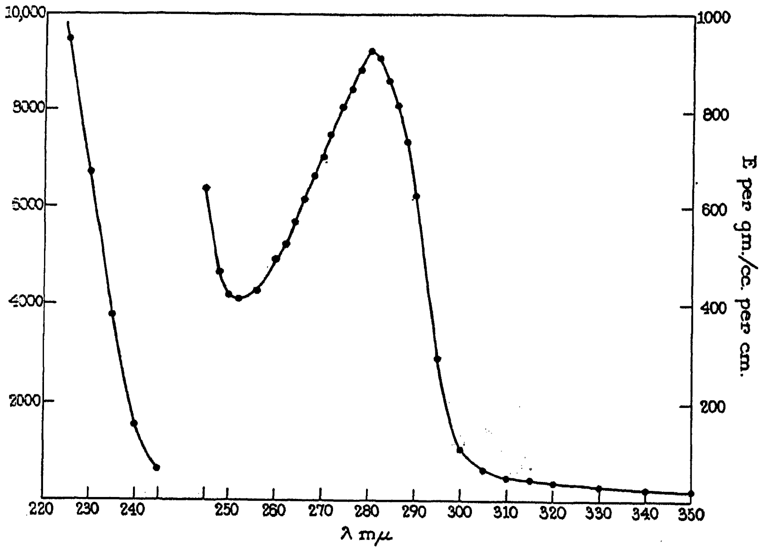


FIG. 2a. Absorption spectrum of horse apoferritin. The ordinate represents the extinction coefficient

$$E = \log_{10}\frac{I_0}{I}\cdot\frac{1}{cl}$$

where the concentration, c, is in grams of protein per cubic centimeter and the light path, l, is 1 cm. The curve at the left has units of extinction 10 times that at the right.

5. Magnetic susceptibility of ferritin iron

In 1943 Michaelis *et al.* (32, 33) discovered that ferritin iron, which is in the ferric state, could be uniquely characterized by its magnetic susceptibility. There are three possible types of ferric atoms containing, respectively, either five, three, or one unpaired electrons per iron atom. It is the three-unpaired-electron type of ferric atom which is characteristic of ferritin, and ferritin is the only biological iron compound of this type occurring naturally in the animal organism.

A ferric compound may be considered, in general, to be an octahedral complex, the iron atom being located in the center of the octahedron and bonded by six atoms or atom groups at the corners of the octahedron. A ferric ion possesses twenty-three planetary electrons, five of these electrons being present in its outermost 3*d* subshell. In the formation of ferric compounds, three cases arise, according to the treatment developed by Pauling (42). The five electrons may remain unpaired as in $FeF_3 \cdot 3H_2O$; here all the bonds to the central iron atom are ionic or ion-dipoles. Or these five electrons may pair, leaving only one unpaired electron as in $[Fe(CN)_6]^{---}$; here all the bonds to the central iron atom are covalent. Or the intermediate case may arise infrequently in which two of the five electrons pair, leaving three unpaired electrons as in ferritin iron or as in alkaline ferrihemoglobin; here four of the bonds may be considered to be covalent and two bonds to be ionic.

In a strong magnetic field the unpaired electrons in an iron atom behave like little magnet dipoles, arranging themselves with their spins parallel to each other and tending to increase the magnetic lines of force; the temperature-dependent movement of the atoms tends to disorient this arrangement. The paramagnetic susceptibility of a substance, say per gram-atom of iron, can be determined by measuring the pull exerted by an inhomogeneous magnetic field on a cylinder containing the iron atoms in solution. Correction of this value for the diamagnetic susceptibility of the solvent and for the effect of temperature gives the magnetic dipole moment, ϵ, in Bohr magnetons.

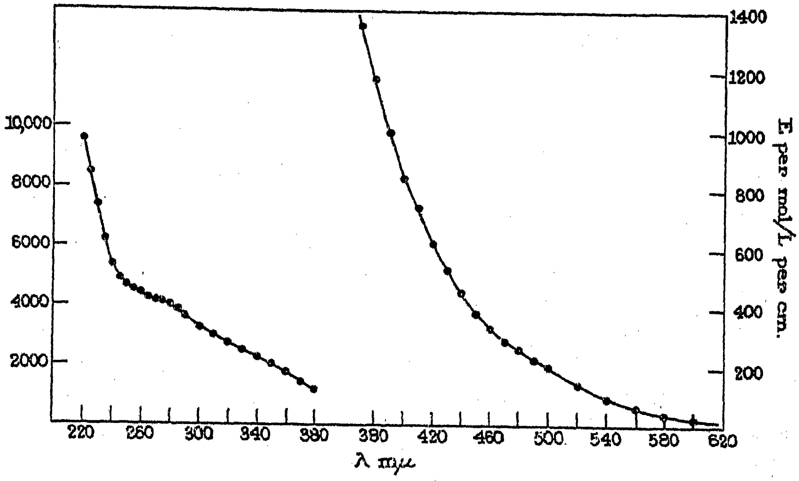


FIG. 2b. Absorption spectrum of horse ferritin. The ordinate represents the molar extinction of ferritin iron, where the concentration of iron is in moles per liter. The curve at the left has units of extinction 10 times that at the right.

The Curie law expresses the temperature dependence of the paramagnetic susceptibility per gram atom, χ, at $T°$ Kelvin. The magnetic dipole moment is

$$\epsilon = 2.84 \sqrt{\chi T} \text{ Bohr magnetons} \qquad (1)$$

Small orbital contributions being neglected and ϵ being assumed due to electron spin only, then the number of Bohr magnetons, ϵ, is related to the number of unpaired electrons, n, by equation 2.

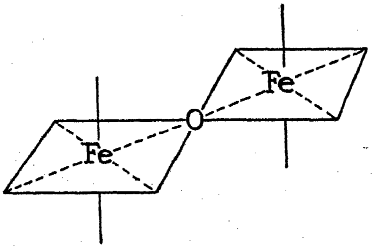
$$\epsilon = \sqrt{n(n+2)} \qquad (2)$$

The Curie law has been shown to be obeyed with sufficient accuracy by ferritin iron within the temperature range 275–301°K., so that the magnetic dipole moment can be used to determine the number of unpaired electrons in ferritin from equation 2. The magnetic dipole moment per iron atom of ferritin is 3.8 Bohr magnetons, a value which would be expected from the spins of three unpaired electrons alone, without any additional contribution from the orbital motions. Such a value occurs rarely for iron compounds, and might be attained by an appropriate mixture of types of iron atoms containing one and five unpaired electrons. However, the constancy of the magnetic dipole moment for various preparations of ferritin is a strong argument in favor of the homogeneity of ferritin iron. The value for the dipole moment remains the same even after the iron hydroxide micelles are precipitated from the protein by treatment with alkali and dialyzed.

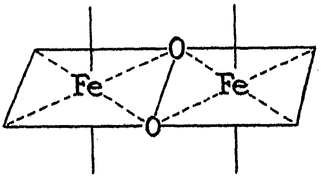
In a study of the magnetic susceptibility of various iron hydroxides, especially in the form of colloidal solutions, a large variability was found. Under specified conditions one could obtain iron hydroxides approaching either the one- or the five-unpaired-electron type per iron atom, and various gradations between them, but there was no way found to make a uniform iron hydroxide containing three unpaired electrons per iron atom. Suspensions of hemosiderin granules were found to have an apparent magnetic moment of 3.4 Bohr magnetons, a value distinctly below that for ferritin.

The relationship proposed by Michaelis between magnetic susceptibility values for iron and the bonding angles suggests the use of magnetic measurements in the structural interpretation of colloidal ferric oxides. The solubility of individual $Fe(OH)_3$ or $Fe(OH)_3 \cdot 3H_2O$ units is extremely small, roughly about 10^{-17} moles per liter. This figure indicates the instability of such a molecule and its small probability of existence as a unit. The instability is due to a great tendency for two hydroxyl groups of adjacent octahedral complexes to split out water molecules, producing Fe—O—Fe bridges and giving rise to polymers which represent the "micelles" of colloidal ferric hydroxide. Alternately, two hydroxyl groups within one molecule of ferric hydroxide may split out water, producing oxygen doubly bound to iron (i.e., Fe=O).

Two octahedral complexes may be considered to combine by sharing either a corner (I) or an edge (II) of the octahedra. The two bonds of the bridge oxygen in I (Fe—O—Fe) form an angle of approximately 180° with the oxygen,



I



II

a condition which can only arise if the bonds are ionic. In II the octahedra share an edge, the bond angles of Fe—O—Fe being compatible with the assumption that these bonds are covalent. In addition to dehydration processes, hydrogen bonding will increase the tendency toward polymerization, the hydrogen bonds forming between adjacent =O and —OH groups. From these considerations the complexity of structure and bonding of various of the colloidal iron hydroxides becomes understandable, and makes more likely the assumption that the ferric hydroxide micelles of ferritin are built up not haphazardly but according to a definite pattern which cannot as yet be reproduced in the laboratory.

D. CHEMICAL PROPERTIES

1. Analysis of ferritin

Chemical analyses of various crystalline ferritin samples (9, 25) for iron, phosphorus, and nitrogen indicate their inhomogeneity (table 1). This is compatible with the fact that ferritin consists of apoferritin molecules containing variable amounts of colloidal iron hydroxide–iron phosphate micelles. In these analyses it is necessary to correct for the rather high cadmium content present even in well-dialyzed ferritin solutions. The cadmium may amount to 1–3 per cent of the dry weight of the crystals. In several different preparations of horse spleen the iron content varied from 18 to 23 per cent and the phosphorus ranged from 1.26 to 2.0 per cent.

TABLE 1

*Analyses of non-hemin iron components of horse spleen in per cent of dry weight**

PREPARATION	Fe		Cd	P	N
	Uncorrected for Cd	Corrected for Cd	Uncorrected for Cd		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Ferritin crystallized once	22.6	23.1	1.93	1.99	12.6
Ferritin crystallized four times	19.7	20.2	2.74	1.42	11.1
Fraction precipitated with Na_2SO_4 (not crystallized)	24.5			1.45	11.0
Hemosiderin granules	8.29			1.59	12.9
"Non-crystallizable ferritin" from mother liquor	19.8	20.2	2.02	1.52	9.12

* All preparations were dried in thin layers at 80°C. for 24 hr. and then for 3 hr. at 110°C.

2. Analysis of apoferritin

The dialyzed protein apoferritin (16) is chemically quite homogeneous, various samples giving the same analytical results. The data are representative of a typical protein: nitrogen, 16.1 per cent; sulfur, 0.97 per cent; carbon, 51.3 per cent; hydrogen, 7.09 per cent; the value for cadmium is 0.73 per cent. On acid dialysis of ferritin, the micelles go into solution but the denatured protein remains in the dialysis bag. Kuhn *et al.* (22) reported the composition of this denatured protein to be: nitrogen, 16.1 per cent; sulfur, 0.88 per cent; carbon, 49.3 per cent; hydrogen, 7.14 per cent. As would be expected, these values are almost identical with those of crystalline apoferritin.

Because of the relative insolubility of apoferritin in electrolyte-free water, it has been possible to recover 84 per cent of ferritin nitrogen as apoferritin. If the nitrogen remaining in the mother liquors from the crystallizations is also included, this would make a recovery of 92 per cent of the nitrogen. This high recovery, considering losses in manipulation and denaturation, makes it unlikely that a nitrogenous compound will be found to be a constituent of the iron micelles themselves, although this possibility cannot be ruled out entirely by the data at hand.

The amino acid compositions of apoferritin as recorded by Kuhn *et al.* (24) and by Tria (53) are at marked variance with one another and will therefore not be tabulated. Archibald (1), using an arginase method for the determination of arginine, has analyzed a sample of dialyzed crystalline apoferritin and found it to contain 8.28 per cent of arginine. The figures of Kuhn *et al.* (24), recalculated in terms of apoferritin, would give about 16 per cent arginine.

3. Composition of ferritin–iron micelle

The iron micelles may be removed by denaturing ferritin with 1 *N* alkali for 10 min. at room temperature. A red-brown coagulum precipitates which is centrifuged down, washed, and dialyzed. As noted above, the magnetic susceptibility of this preparation per iron atom is identical with the magnetic susceptibility per iron atom found for ferritin. However, alkaline treatment has removed about three-fourths of the phosphorus originally attached to the iron micelles, probably replacing an $—OPO_3H_2$ group by an $—OH$ group. Some nitrogen is also found in this preparation of alkaline-treated micelles. This is assumed to be due to the occlusion of 10 per cent by weight of the denatured protein. Assuming the nitrogen as impurity, it is concluded from the analyses that the ferritin micelles have the approximate composition $[(FeOOH)_8 \cdot (FeOPO_3H_2)]$ (14).

The evidence for phosphorus being a constituent of the iron micelles is that on treatment of ferritin with acid or alkali and subsequent dialysis there remains a denatured protein devoid of phosphorus. Likewise, treatment of ferritin with dithionite to remove the iron micelles, leaving the protein undenatured, results in an apoferritin containing no phosphorus. The claim of Kuhn *et al.* (24) that phosphorus was a constituent of a nucleic acid which formed 12.1 per cent by weight of ferritin could not be substantiated (9).

The phosphorus of the micelles does not appear to be present merely adsorbed to the surface of the micelles, since it cannot be displaced by ions like citrate, nor are appreciable amounts of radio P^*O_4 adsorbed by either ferritin or apoferritin (14). The PO_4 is not only chemically bound on the surface of the micelle but is distributed throughout the micelle. This conclusion was arrived at by studying the change in the ratio of iron to phosphorus in ferritin when treated with ammonium magnesium citrate during a several-day period. Treatment at room temperature with *N* hydrochloric acid releases the phosphate from the micelles within a few minutes, and all of the phosphorus can be accounted for as orthophosphate. All this evidence supports the view that the iron micelle in ferritin is a basic ferric phosphate, where some of the —OH in FeOOH is replaced by $—OPO_3H_2$ groups.

There is as yet no good way of determining the size of the iron micelles, nor has the specific attachment of the micelles to the apoferritin been investigated.

E. DISTRIBUTION OF FERRITIN

1. *Ferritin in species and organs*

The isolation of ferritin from a number of vertebrate species was accomplished by using the identical method used for the isolation of horse spleen ferritin. In the order of decreasing ferritin content (table 2) the species are the horse, man, guinea pig, mouse, rat, rabbit, and cat (10). Kuhn *et al.* (24) also reported the isolation of ferritin from the jackal. From 100 g. fresh weight of horse spleen one may obtain as much as 0.1 g. dry weight of crystalline ferritin. No crystalline ferritin could be obtained from the liver or spleen of beef cattle, sheep, deer, various species of fowl, various species of fish, and bullfrogs. This failure to isolate crystalline ferritin from these species may in part be due to the variations in the properties of the protein.

TABLE 2

Ferritin distribution in animal species and organs as determined by isolation procedure (10)

SPECIES	ORGANS AND TISSUES EXAMINED			
	Spleen	Liver	Red bone marrow	Other organs in which ferritin was found
Horse	+++++ (0.25–0.025)*	+	+++	Testes† +
Human	++	+++ (0.23–0.006)	++	
Dog	+	++ (0.039–0.000)	0	Kidney + M
Guinea pig	+	++ (0.060–0.015)	0	
Mouse	+	+		Testes† +
Rat	+	+		
Pig	+	+		Testes† +
Rabbit	+	0	0	Testes† +
Cat	0	0		Kidney +

* The figures in parentheses represent the extremes of ferritin found in these organs, expressed in terms of the number of cubic centimeters of packed, twice-crystallized ferritin crystals per 100 g. of fresh weight of the organ. One cubic centimeter of packed crystals, dried at 110°C., weighs approximately 0.40 g.

† The crystals obtained from the testes of the horse, mouse, pig, and rabbit are either colorless or very pale yellow.

In those species from which crystalline ferritin could be isolated it was found that it could also be made to appear in a teased preparation under the microscope. A piece of liver or spleen about 2–3 mm. thick and 5 mm. square was teased slightly in a drop of 10 per cent cadmium sulfate solution on a slide, a cover slip was placed on the preparation, and it was placed in a moist chamber to prevent evaporation. Within half an hour, or overnight, crystals of ferritin appeared, especially in small areas where the tissue folds had entrapped cadmium sulfate. The crystals were readily distinguished from the brownish hemosiderin granules; in the human tissue preparations the crystals generally appeared as spheroids, making a distinction from hemosiderin less clear.

The organs from which crystalline ferritin could be isolated are the spleen, liver, bone marrow, and kidneys. Very pale yellow or colorless crystals were isolated in small yield from the testes of several species. There were wide individual variations in the content of ferritin from livers and spleens of the same species (10). Recently, ferritin has been detected in the mucosa of the gastrointestinal tract of the guinea pig. The significance of this finding is discussed below.

2. *Antigenic studies and cross reactions*

Antigenic studies and cross reactions of ferritin have been made by preparing an antibody in the rabbit against horse ferritin (10). As was to be expected, the antibody is directed against the protein apoferritin and the precipitin reaction is not hindered by the presence of the iron micelles. By using the precipitin reaction as a test for apoferritin in the different organs of the horse, apoferritin was detected in the spleen, bone marrow, liver, testicle, kidney, adrenal, pancreas, ovary, and lymph node. Its presence in the brain was uncertain. Apoferritin was absent from blood, striated muscle, stomach mucosa, and pituitary.

Apoferritin is of interest from the immunological point of view, since it represents the first clear-cut example that the pattern of a protein of one tissue is constructed to be immunologically identical with the same protein found in another tissue. There appears to be no evident relation between the distribution of apoferritin in the various tissues and the origin of these tissues from the basic embryonic germ layers. Horse apoferritin antibody cross reacts and gives a weak precipitin reaction with dog apoferritin. There is no cross reaction between horse apoferritin antibody and human apoferritin.

F. MICROCHEMICAL TESTS FOR IRON IN TISSUES

1. *Methods*

Iron distribution in tissues has been studied extensively by histologists, although a critical analysis of the techniques involved is often lacking. Several methods have been used which depend either upon the formation of an insoluble ferric compound in acid solution, for example, Prussian blue, or the formation of an insoluble ferrous compound in neutral or alkaline solution, for example, ferrous sulfide. They all have one defect: they require that iron be in a monomolecularly dispersed form before it can form a visually detectable insoluble compound. During the interval between dispersal and the reaction to form an insoluble compound the iron may become adsorbed preferentially to some cellular structures which normally would not contain iron, thus leading to erroneous observations. Especially is there danger of this happening in the procedures claimed to unmask iron from organic compounds, such as hemin, by pretreatment with strong acid (39). In order to minimize this possibility, the reaction solubilizing the iron should be followed very rapidly by the reaction precipitating the iron again in a visually discernible form.

In the ferrocyanide method the inorganic iron compounds are dissolved to form ferric iron and react with ferrocyanide to form the insoluble Prussian blue. In the sulfide method it has been customary to use ammonium sulfide, the ferric iron being reduced to the soluble ferrous iron and then forming the greenish black insoluble ferrous sulfide. The use of acid or ammoniacal solutions generally requires a previous sojourn in a fixing fluid, the assumption being made that the fixing fluid has not changed the iron distribution. If no fixing fluid has been used,—as, for example, in dried blood smears,—then the acid or alkaline solution will dissolve out or coagulate proteins. Obviously, a method which can be applied to the living cell without distorting the protoplasm would be desirable. This can be achieved very simply by using a saline solution through which hydrogen sulfide has been freshly bubbled for several minutes. The hydrogen sulfide in approximately neutral solution reduces even basic ferric hydroxide to the ferrous condition and forms the very insoluble green-black ferrous sulfide (11).

2. Iron in horse spleen

Even by using this delicate hydrogen sulfide method it has not been possible to determine the localization of ferritin in particular cells (11). The fresh horse spleen tissue which is richest in ferritin after hydrogen sulfide treatment showed large black hemosiderin granules in the macrophages, tiny discrete black granules on some crenated erythrocytes, and pale grey staining erythrocytes indicating a diffuse distribution of iron in these cells. It was not possible to decide whether this diffusely staining iron in the erythrocytes represented ferritin, but it is unlikely that a cell in the process of destruction, as it is assumed these cells are in the spleen, would build a new protein. Rather it is plausible to assume ferritin to be so diffuse that no simple microscopic observation of iron distribution could demonstrate particular cells in which ferritin was present.

3. Consideration of iron in nucleoprotein

From time to time the presence of iron associated with nucleoprotein has been reported (39, 46, 58). Macallum (29), using prolonged treatment with ammonium sulfide, claimed that the chromatin became specifically darkened. Okamoto (40), studying various tissues of vertebrates and invertebrates, also used prolonged treatment with acid and ferrocyanide and reported iron to be preferentially localized in the nuclei. Saha and Guha (45) reported the isolation of nucleoproteins containing both iron and copper. The ease with which nucleic acid binds iron is attested by the common use made by histologists of iron alum as a mordant for chromosome staining. The papers suggesting that tissue extracts contain the iron bound to nucleoprotein have dealt with mixtures, and no conclusions may be drawn from them. Nor has the idea (24) that ferritin contains nucleic acid been confirmed. As for nucleoprotein itself being rich in iron, we have examined two preparations of nucleoprotein. These were isolated by A. E. Mirsky from sheep and calf spleen nuclei, using very mild treatment with sodium chloride solutions and differential centrifugations to make the separations (34). These preparations contained 0.036 per cent and 0.007 per cent of iron, respectively, on a dry weight basis (13), i.e., negligibly small concentrations of iron. One may say categorically that no satisfactory evidence exists today which supports the view that iron is localized preferentially in nuclei or with nucleoproteins.

G. POSSIBLE FUNCTION OF FERRITIN AS A MUCOSAL REGULATOR OF IRON ABSORPTION

Recent studies have brought to light a number of interesting facts which govern the process of iron absorption. The iron is apparently absorbed in the ferrous state (23, 27, 28, 35, 52, 56), various portions of the gastrointestinal tract being capable of absorbing it (3, 7, 20).

In 1937 Widdowson and McCance (30, 57) presented evidence that iron once absorbed or injected parenterally remained in the body, no significant amounts being excreted. Evidence on the clinical side by Welch *et al.* (56) also supported this idea. Using radioactive iron as a tracer, Hahn, Bale, Lawrence, and Whipple (19), in a fundamental experiment, came to the conclusion that the rate of absorption of iron was probably governed by some process in the gastrointestinal mucosa. Further studies from Whipple's (4, 20) laboratory firmly established the fact that the total iron content of the body was not controlled by unlimited absorption and excretion of excess as are the common elements, but rather by limiting the intake of iron.

Two experiments clearly revealed the regulatory process of the mucosa (20). A dog, when suddenly made anemic and simultaneously fed radio iron, absorbed no more iron at this time than did a normal dog. The body stores were gradually depleted, and within 7 days iron absorption had increased to 10–20 times the normal rate. In another experiment a chronically anemic dog was fed a 10-mg. dose of iron and after 1–6 hr. was fed a 10-mg. dose of radio iron. The radio iron was found to be absorbed at a much lower rate than was the first dose. These experiments indicated that a "mucosal block" or saturation, with respect to iron, could be built up within a few hours and be maintained for several hours to several days, appreciably diminishing iron absorption. The lag in iron absorption, Hahn *et al.* suggested, might be due to a "mucosal acceptor" having the properties of apoferritin which could accept the iron and which when saturated would prevent any further uptake of iron. We should like to modify this hypothesis by considering the mucosal cells to be "physiologically saturated" with respect to ferrous ions rather than with respect to ferritin.

According to this hypothesis the regulation of iron absorption may occur in the following way: Iron entering the mucosal cells would be converted to ferritin iron. This ferritin iron would be in equilibrium with small amounts of ferrous ions in the cells, and the ferrous ions would be in equilibrium with the plasma Fe^{+++} iron of the blood stream (figure 3). The creation of an anemic condition would result in draining the storage iron primarily from the marrow, spleen, and liver. Only when this major source had been depleted and the plasma iron level had been lowered would iron from the mucosa begin to move out into the blood stream. The "physiological saturation" of the mucosal cells with respect to ferrous ions would, however, be maintained until part or all of the ferritin iron had been converted to ferrous iron. Only then would the ferrous iron fall below its "saturation" value in the mucosal cells, and when this occurred radioactive iron would begin to be absorbed.

Recently (12) evidence was found which appears to support the hypothesis of the regulatory action of ferritin in iron absorption. When about 0.2 g. of the intestinal mucosa of the guinea pig was scraped off onto a slide, mixed with 10 per cent cadmium sulfate, and allowed to concentrate by slow evaporation overnight in a moist chamber, crystals of ferritin were found. By counting the number of crystals per slide it was possible to determine in a semiquantitative way the distribution of ferritin in 2–5 cm. segments along the intestinal tract.

In rapidly growing guinea pigs of 400 to 600 g. barely detectable amounts of ferritin (1–10 tiny crystals per slide) were observed and here only in the mucosa of the duodenal region. When 20 mg. of iron was fed in the form of ferrous ammonium sulfate, an increase in ferritin was observed within 4 hr. after feeding (50–100 crystals per slide for the duodenal region), and the maximum amount of ferritin was reached some 8 hr. after feeding (100–500 crystals per slide for the duodenal region). Twenty hours after iron feeding, ferritin was occasionally detected in the cardiac region of the stomach; it was generally present in the pyloric region (10–50 crystals per slide); it was highest in the duodenal region (100–500 crystals per slide), i.e., in a 5-cm. segment below the pyloric sphincter; it diminished along the jejunum (50–100 crystals per slide) and ileum (10–50 crystals per slide); it was occasionally detected in the cecum; it was low in the upper portion of the large intestine (0–10 crystals per slide); and it was occasionally detectable in the lower portion of the large intestine. Some 5 to 7 days after iron was fed, the ferritin content had diminished considerably and was almost down to the concentration present in the mucosa of the control guinea pigs.

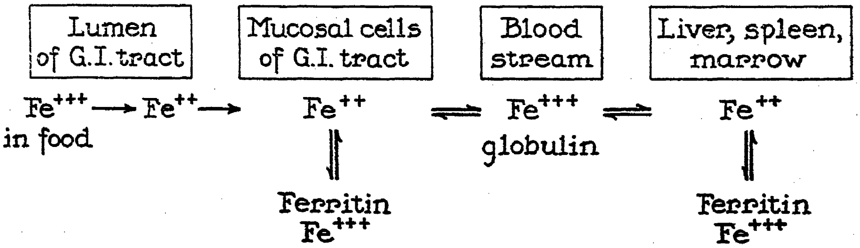


FIG. 3. Scheme representing the rôle of ferritin in the absorption and storage of iron.

Of all the organs and tissues of the guinea pig which were examined, it was found that per unit weight the liver and duodenal mucosa were highest in ferritin. This relatively high content of ferritin in the mucosa, the properties of ferritin as an iron-storage compound, the rapid increase of ferritin in the mucosa in response to iron feeding, the diminution of ferritin in the mucosa several days afterwards, paralleling the phenomena of the "mucosal block",—all of these factors are in keeping with the hypothesis of ferritin functioning in the regulation of iron absorption by the mucosa.

H. IRON TRANSPORT IN THE BLOOD STREAM

Once the ferrous iron has passed through the mucosa into the blood stream (36), the oxygen tension is sufficient to autoxidize immediately any ferrous iron to the ferric state. The ferric iron, probably in the form of ferric hydroxide, then attaches avidly to the serum proteins (49, 50). It should be emphasized at this point that ferritin itself is not transported through the blood, since even the very sensitive precipitin reaction has not revealed any ferritin in blood (10).

The concentration of ferric iron in plasma averages about 100–200 mg. per 100 cc. of plasma (54). Little is known of how this relatively steady state is maintained. The following picture is suggested: Ferritin in the cells may be considered to be in equilibrium with a small amount of ferrous iron in the cells, the ferrous iron being capable of transport to the blood stream. One of the important factors governing the level of ferrous iron in the cells is the rate of autoxidation of ferrous to ferric ions and the rate of reduction of ferritin iron to the ferrous condition. The level or steady state of the serum iron would then be governed, in part at least, by what one may loosely term the "redox level" of the storage tissues.

As tracer studies indicate (14), large amounts of iron in the ferric state can be taken out of circulation within a few hours by the liver, some going directly to the bone marrow. Here also we are ignorant of how iron is transported from the blood stream into the liver cells, for example. It is interesting to note that the tissues which take out iron most avidly from the blood stream are those which contain numerous phagocytes of the macrophage type. The possibility suggests itself that it is these phagocytes which might ingest the serum protein iron complexes, reducing the iron, and passing it on to adjacent cells where conversion to ferritin might occur.

I. FUNCTION OF FERRITIN AS AN IRON-STORAGE COMPOUND

The chemical and physical properties presented above make it apparent that ferritin would be an ideal substance for storage both of iron and of protein. Ferric ions are rather toxic to protoplasm, tending to coagulate proteins and enzymes. In ferritin the iron is stored in the form of colloidal micelles or aggregates of very insoluble basic iron phosphate attached to a relatively large non-diffusible protein molecule, apoferritin. When iron is required for heme synthesis it is made rapidly available. A reasonable hypothesis is that the iron of the micelles is made available by reducing the iron to the relatively soluble ferrous form. At the same time demands for globin synthesis would seem to be met in part by breaking down the apoferritin molecule itself (10).

1. *Ferritin in anemic and in normal horses*

One of the observations to support the idea that ferritin is an iron-storage compound arose accidentally from a study of horse spleens obtained from the Antitoxin Laboratories at Otisville, N. Y. (10). Horses which had been bled frequently for serum were found to have spleens from which relatively little ferritin could be obtained, in contrast to normal horse spleens which had a high ferritin content. Not only was ferritin iron and total iron low in bled-horse spleens, but the protein apoferritin was also low. This was interpreted as showing that in anemic animals there was a drain not only of iron but also of apoferritin and probably of other proteins to meet the demands of rapid blood formation.

2. *Conversion of tagged hemoglobin iron to ferritin iron*

A more direct approach to the problem of the function of ferritin was made by Hahn, Granick, Bale, and Michaelis (21), using radioactive iron as tracer. In order to determine whether hemoglobin iron could be converted into ferritin iron, a donor dog was prepared. The donor dog was first made anemic by bleeding. Then the animal was injected with a radioactive iron solution of ferric ammonium citrate, at the same time that it was fed liver. A rapid synthesis of red cells occurred which contained the radio iron as a constituent of the heme in hemoglobin. Of this donor dog's blood 110 cc. was injected intravenously into a recipient dog (table 3). On the following day the recipient dog received a subcutaneous injection of acetylphenylhydrazine to increase the destruction of the red cells. After 6 days the dog was sacrificed. Of the radioactive iron lost from the circulation 55 per cent was found in the liver and 20 per cent in the spleen. About half of the total iron of the liver was isolated in the iron-rich fraction (F + NCF); one out of every four atoms of this iron was radioactive. Crystalline ferritin prepared from this fraction also contained the same propor-

TABLE 3

Conversion of tagged red cell hemoglobin iron to ferritin iron (21)

	DISTRIBUTION OF LABELED IRON		PER CENT OF IRON IN THE TISSUES WHICH IS RADIOACTIVE
	Total circulating activity after transfusion taken as 100 per cent	Activity lost from circulation taken as 100 per cent	
	per cent	*per cent*	
Injected tagged blood (110 cc.)	100		
Circulating blood after transfusion	100		19.8
Circulating blood before death	15.6		7.9
Liver (335 g.)	46.1	55	21.4
F + NCF			25.3
Ferritin			25.5
Spleen (89 g.)	17.1	20	12.5
F + NCF			12.0

tion of radioactive iron to total iron as did the ferritin-rich fraction. This experiment therefore demonstrates the conversion of heme iron of the red blood cells into iron which can be isolated as a constituent of crystalline ferritin.

3. *Conversion of tagged inorganic iron to ferritin iron*

In another experiment (21) it was demonstrated that radio ferric ammonium citrate could be injected intravenously into a dog and be converted to ferritin iron of a magnetic susceptibility characteristic of normal ferritin. Depending on the method of preparation, ferric ammonium citrate can be obtained with various magnetic susceptibilities. Radio ferric ammonium citrate prepared in a slight excess of ammonium citrate (14) has a magnetic moment of 5.69 B.U. When this compound is mixed *in vitro* with dog plasma, little change magnetically can be detected in half an hour.

Some 13 days after intravenous injection of radio ferric ammonium citrate the dog was viviperfused. Over 80 per cent of the radioactive iron was found to be present in the liver, only a trace being present in the spleen. The iron-rich fraction of the liver (F + NCF), making up about 25 per cent of the radioactive iron injected, was subjected to magnetic measurements. The magnetic moment was 3.78 B.U. per gram-atom of iron, which is the same value as that found for all previous samples of ferritin. The iron-rich fraction was further treated to isolate crystalline ferritin; this also had a radioactivity equivalent to that of the (F + NCF) fraction, i.e., about 75 per cent of the iron was radioactive iron. It was thus shown that inorganic ferric iron of a magnetic susceptibility representing approximately five unpaired electrons per iron atom could be converted into a basic iron phosphate of magnetic susceptibility representing three unpaired electrons per iron atom and could be isolated as a crystalline compound, ferritin. Here again the storage function of ferritin was clearly evident.

4. *Rate of conversion to ferritin iron*

In order to find out how rapidly the conversion of inorganic to ferritin iron occurred, three dogs were sacrificed 1, 2, and 5 hr. after intravenous injection with radioactive ferric ammonium citrate (14). One hour after injection 40 per cent of the radioactive iron was localized in the liver, and 2 hr. after injection 61 per cent was found in the liver. The iron-rich fraction (F + NCF) had the red-brown color and paramagnetic susceptibility characteristic of ferritin. Derangement of ferritin synthesis was suggested by the results obtained with the third dog, sacrificed after 5 hr. The liver of this dog had the ability to take up radioactive iron, since 40 per cent of the radioactive iron injected was found in the liver. However, the fraction (F + NCF) which was isolated did not possess the normal red-brown color of ferritin but was rather of a pale brownish yellow color. Its magnetic moment was 4.7 B.U. instead of the normal 3.8 B.U.; nor could ferritin crystals be isolated from this fraction. The experiment seems to indicate that in this particular case, although the liver could take up iron from circulation, it could not convert it into ferritin iron.

J. SOME FACTORS IN FERRITIN FORMATION

For ferritin formation not only should the cell be able to produce apoferritin but it must have a mechanism for accumulating iron and possibly another for attaching iron to apoferritin. These conclusions are suggested by the observation that apoferritin, but little or no ferritin, is present in testicles (10). It may be that the mechanism accumulating iron or forming micelles is not present, or is present to a very slight extent in testicular tissue. Also, the liver of the abnormal dog mentioned above could take out much iron readily from the blood stream but was unable to convert it to normal ferritin, perhaps because the protein, apoferritin, was lacking. In connection with the quantity of apoferritin present, it should be mentioned that apoferritin may serve as a protein reserve, probably along with other proteins, to be used for the synthesis of new erythrocytes when there is a demand for it or during starvation.

Recently (12) a marked increase of ferritin in the gastrointestinal mucosa was observed in response to iron feeding. When ferrous ammonium sulfate was fed to guinea pigs, the ferritin especially of the duodenal mucosa was greatly increased. No apoferritin crystals could be detected before iron feeding, indicating that apoferritin was not originally present in any appreciable concentration. These facts suggest that the feeding of iron has in some way brought about an increase in the concentration of a particular protein which combines with iron to form a storage compound of iron in the mucosa.

Ferritin formation appears to be a result of a biological mechanism. *In vitro* experiments negate the possibility that ferritin or apoferritin accumulates iron merely by a kind of crystal growth in the presence of either ferrous or ferric iron. Guinea pig liver mash, when mixed and incubated with apoferritin and radio iron, appeared to bring about the conversion of inorganic iron to ferritin iron, indicating that some specific biological mechanisms were functioning (14). The extent of this conversion was, however, very low.

An "apparent" synthesis of ferritin occurs if one mixes apoferritin with non-crystallizable ferritin and adds cadmium sulfate (16). The crystals resulting are brown, contain iron, and are indistinguishable from ferritin. We believe that this does not represent an addition of iron micelles to apoferritin molecules but rather that the apoferritin lattice is sufficiently porous to permit certain ferritin molecules in the non-crystallizable ferritin solution (9) to fit into it.

K. SUMMARY OF IRON METABOLISM IN RELATION TO FERRITIN

Figure 3 is an attempt to summarize the facts and hypotheses of iron absorption, transport, storage, and function. Iron enters the gastrointestinal tract in the ferric state with the food. It is converted to the ferrous state with the aid of acidity, —SH groups, ascorbic acid and possibly other reducing agents of the food. Absorption of ferrous iron occurs mainly into the mucosal cells of the duodenum and jejunum, and appears to be unidirectional. In response to iron feeding the protein apoferritin increases in concentration in the mucosal cells so that more ferritin accumulates in these cells. The mucosal cells regulate iron absorption, it is postulated, by maintaining within the cells a level of ferrous iron, governed in part by the redox level of these cells. The ferrous iron of the mucosal cells is further postulated to be in equilibrium with the ferritin in the mucosal cells and with the plasma iron of the blood stream. From the mucosa the ferrous iron moves into the blood stream, where it is at once autoxidized to ferric hydroxide, which is then adsorbed to the serum proteins and is transported as a ferric hydroxide–protein complex. According to these hypotheses a lowering of the plasma iron would result in more rapid movement of iron out of the mucosal cells, depleting the stores of ferritin iron and finally lowering the concentration of ferrous iron in the mucosal cells below the "physiological saturation" level. At this time, then, increased absorption of iron from the gastrointestinal tract would be observed.

The plasma protein iron is in equilibrium and exchanges rapidly with the ferrous iron and ferritin of the liver, spleen, and bone marrow. Here also the ferritin is in equilibrium with ferrous iron, only ferrous iron being suggested as capable of transfer from the cells to the blood stream. In the marrow not only is ferrous iron converted to ferritin, but ferrous iron is also incorporated into protoporphyrin to form hemin, and the hemin is attached to globin to form hemoglobin. Damaged red cells are destroyed by the phagocytes of the liver, spleen, and marrow. The iron released from hemoglobin breakdown, unlike the porphyrin moiety, is carefully conserved by the body to be re-used.

We should like to take this opportunity to thank Dr. Leonor Michaelis for his suggestions and advice in these studies.

V. REFERENCES

(1) ARCHIBALD, R. M.: Unpublished work.
(2) ASHER, TH.: Z. physiol. Chem. **220**, 97 (1933).
(3) AUSTONI, M., AND GREENBERG, D.: J. Biol. Chem. **134**, 27 (1940).
(4) BALFOUR, W., HAHN, P., BALE, W., POMMERENKI, W., AND WHIPPLE, G.: J. Exptl. Med. **76**, 15 (1942).
(5) BUROW, ROBERT: Biochem. Z. **25**, 165 (1910).
(6) COOK, S.: J. Biol. Chem. **82**, 595 (1929).
(7) COPP, H., AND GREENBERG, D.: Federation Proc. **4**, 86 (1945).
(8) FANKUCHEN, I.: J. Biol. Chem. **150**, 57 (1943).
(9) GRANICK, S.: J. Biol. Chem. **146**, 451 (1942).
(10) GRANICK, S.: J. Biol. Chem. **149**, 157 (1943).
(11) GRANICK, S.: Proc. Soc. Exptl. Biol. Med. **53**, 255 (1943).
(12) GRANICK, S.: Science **103**, 107 (1946).
(13) GRANICK, S.: Unpublished work.
(14) GRANICK, S., AND HAHN, P.: J. Biol. Chem. **155**, 661 (1944).
(15) GRANICK, S., AND MICHAELIS, L.: Science **95**, 439 (1942).
(16) GRANICK, S., AND MICHAELIS, L.: J. Biol. Chem. **147**, 91 (1943).
(17) GRÜNEBERG, H.: Nature **148**, 114 (1941).
(18) HAHN, P.: Medicine **16**, 249 (1937).
(19) HAHN, P., BALE, W., LAWRENCE, E., AND WHIPPLE, G. H.: J. Exptl. Med. **69**, 739 (1939).
(20) HAHN, P., BALE, W., ROSS, J., BALFOUR, W., AND WHIPPLE, G. H.: J. Exptl. Med. **78**, 169 (1943).
(21) HAHN, P., GRANICK, S., BALE, W., AND MICHAELIS, L.: J. Biol. Chem. **150**, 407 (1943).

(22) Heath, C.: *Symposium on Blood*, pp. 41–51. Wisconsin Press, Madison, Wisconsin (1939).

(23) Henderson, V., and Lucas, G.: Am. J. Digestive Diseases Nutrition **11**, 244 (1944).

(24) Kuhn, R., Sorensen, N., and Birkhofer, L.: Ber. **73B**, 823 (1940).

(25) Laufberger, V.: Bull. soc. chim. biol. **19**, 1575 (1937); Compt. rend. du XV Congress des Physiologie à Leningrad, p. 232 (1935).

(26) Libet, B., and Elliott, K.: J. Biol. Chem. **152**, 613 (1944).

(27) Lintzel, W.: Ergeb. Physiol. **31**, 844 (1931).

(28) Lintzel, W.: Biochem. Z. **263**, 173 (1933).

(29) Macallum, A. B.: Proc. Roy. Soc. (London) **B50**, 277 (1891).

(30) McCance, R., and Widdowson, E.: Lancet **2**, 680 (1937).

(31) McMeekin, I., and Warner, R.: J. Am. Chem. Soc. **64**, 2393 (1942).

(32) Michaelis, L.: Chapter on Magnetochemistry in *Physical Methods of Organic Chemistry*, Vol. II, in press. Interscience Publishers, New York.

(33) Michaelis, L., Coryell, C., and Granick, S.: J. Biol. Chem. **148**, 463 (1943).

(34) Mirsky, A. E.: Advances in Enzymol. **3**, 1 (1943).

(35) Moore, C., Dubach, R., Minnich, V., and Roberts, K.: J. Clin. Investigation **23**, 755 (1944).

(36) Müller, Franz: Arch. path. Anat. (Virchow's) **164**, 436 (1901).

(37) Nasse, H.: Sitzung ges. Nat., Marberg (1873), No. 2.

(38) Neumann, E.: Arch. path. Anat. (Virchow's) **111**, 25 (1888).

(39) Nicholson, F.: J. Comp. Neurol. **36**, 37 (1923).

(40) Okamoto, Kozo: Acta Schol. Med. Univ. Imp. Kioto **20**, 413–560 (1937).

(41) Pappenheimer, A., Thompson, W., Parker, D., and Smith, K.: Proc. Soc. Exptl. Biol. Med. **56**, 145 (1944).

(42) Pauling, L.: *The Nature of the Chemical Bond.* Cornell University Press, Ithaca, New York (1939).

(43) Perls, M.: Arch. path. Anat. (Virchow's) **39**, 42 (1867).

(44) Rothen, A.: J. Biol. Chem. **152**, 679 (1944).

(45) Saha, K., and Guha, B.: Nature **148**, 595 (1941).

(46) Salkowski, E.: Z. physiol. Chem. **59**, 19 (1909).

(47) Schmiedeberg, O.: Arch. exptl. Path. Pharmakol. **33**, 101 (1894).

(48) Spatz, H.: Z. ges. Neurol. Psychiat. **77**, 261 (1922).

(49) Starkenstein, E.: Klin. Wochschr. **7**, 218 (1929).

(50) Starkenstein, E.: Arch. exptl. Path. Pharmakol. **172**, 75 (1933).

(51) Starkenstein, E.: *Handbuch der experimentellen Pharmakologie*, Vol. 3, p. 682. J. Springer, Berlin (1934).

(52) Tompsett, S.: Biochem. J. **34**, 961 (1940).

(53) Tria, E.: Chem. Abstracts **37**, 898 (1943); Atti accad. Italia, Rend. classe sci. fis., mat. nat. [7] **1**, 579–89 (1940).

(54) Vahlquist, B.: Acta Paediat. **28**, Supplement V (1941).

(55) Wehrle, H.: Arch. Path. **25**, 514 (1938).

(56) Welch, C., Wakefield, E., and Adams, M.: Arch. Internal Med. **58**, 1095 (1936).

(57) Widdowson, E., and McCance, R.: Biochem. J. **31**, 2029 (1937).

(58) Zaleski, St.: Z. physiol. Chem. **10**, 453 (1886).

THE CHEMISTRY OF THE DIARYL ETHERS

HERBERT E. UNGNADE

Chemistry Department, University of Missouri, Columbia, Missouri

Received October 2, 1945

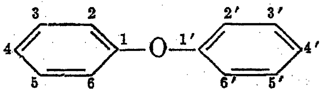
CONTENTS

I. Introduction 405
II. The oxygen valence angle 406
III. Diphenyl ether 406
IV. Alkyldiphenyl ethers 408
V. Aryldiphenyl ethers 409
VI. Phenoxyquinolines and isoquinolines 410
VII. Halogen compounds 410
VIII. Nitrodiphenyl ethers 411
IX. Hydroxydiphenyl ethers 414
X. Alcohols 416
XI. Aldehydes 418
XII. Ketones 419
XIII. Acids 420
XIV. Sulfinic, sulfonic, and seleninic acids 423
XV. Aminodiphenyl ethers 426
XVI. Amino acids 428
XVII. Phosphorus, arsenic, and tellurium compounds 430
XVIII. Mercury, lithium, and sodium compounds 431
XIX. Depsidones 434
XX. Alkaloids 436
XXI. References 440

I. INTRODUCTION

The discussion of diphenyl ethers in this review is limited to those compounds in which two carbocyclic aromatic rings are linked by one oxygen atom. Special emphasis is given to the reactions which are peculiar to such compounds. Other nuclei—carbocyclic or heterocyclic—are regarded as substituents, whether they are fused with one of the "ether" rings or not. Natural products which belong to this category are not specifically treated, unless their reactions are of more general significance to the whole group of compounds.

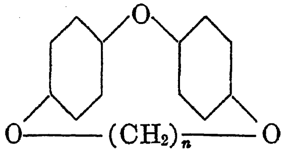
The compounds mentioned are named as derivatives of diphenyl ether when this system is practical; otherwise they are considered as phenoxy derivatives of the appropriate compounds. In *Chemical Abstracts* derivatives are listed under the heading "phenyl ether." For clarity and to avoid ambiguity the prefix di- is retained in the present review. This practice is useful in a discussion of substituted diphenyl ethers, since alkyl phenyl ethers are not the same as alkyldiphenyl ethers. The position of substituents is denoted in the literature by the use of *o*-, *m*-, *p*-, *o'*-, *m'*-, *p'*- and by the following numbering system:



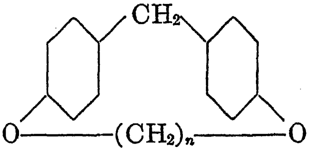
II. THE OXYGEN VALENCE ANGLE

Since oxygen can take the place of methylene and imino groups in cyclic compounds without appreciable change in the stability, strain, and ease of formation of the compounds involved, its valence angle must be similar in magnitude to the tetrahedral angle (178). Reasonably accurate methods give a value of 105° for the oxygen valence angle in water (110, 190). The angle is probably somewhat larger in dialkyl ethers, and apparently variable in size (90a). The determination of the oxygen valence angle in diphenyl ethers from studies of dipole moments has led to divergent values: e.g., 119° or 152° (31), 121° ± 5° (252), 110° (110), 128° ± 4° (265), and 142° ± 8° (111). The method is not free from criticism (131), and the results can be regarded only as approximations. X-ray crystal structure data for *p*,*p*'-diiododiphenyl ether give a valence angle of 118° ± 3° (31).

Lüttringhaus obtained evidence indicative of an angle larger than the tetrahedral angle by comparing the yield curves of the polycyclic systems I and II from the respective ω-bromoalkyl ethers.

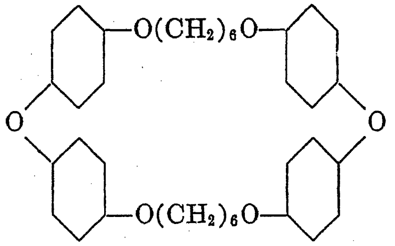


I



II

For $n = 10$ the yields of I and II were 36 and 68 per cent, respectively; for $n = 8$, 0 and 27 per cent; whereas for $n = 6$ the 34-membered ring system (III) was formed.



III

The difference between the valence angles of carbon and oxygen is further indicated by the formation of well-defined eutectics by diphenyl ether and diphenylmethane, their *p*,*p*'-dihydroxy compounds, and the decamethylene ethers (I and II). When the angles are forced to assume the same (or very nearly the same) value, as in fluorene and dibenzofuran, a continuous system of mixed crystals is observed (178) instead of eutectics.

III. DIPHENYL ETHER

Diphenyl ether was prepared in 1845 by List and Limpricht (172) by the dry distillation of copper benzoate. The product was shown to be a mixture of diphenyl ether and diphenyl by Hoffmeister (128), who was the first to isolate the pure substance by separating this mixture. The ether was also prepared by the reaction of benzenediazonium sulfate with phenol (126, 127, 128). The yields were poor in either case. Thus, 5 lb. of copper benzoate gave only 30 g. of purified diphenyl ether. The product was apparently quite pure, since later workers have not succeeded in raising the melting point even after laborious purification (54). Improved yields of diphenyl ether from benzenediazonium chloride were claimed by Hirsch (124, 125), but his claims have not been entirely substantiated (276).

Gladstone and Tribe (89) prepared the ether by the dry distillation of aluminum phenoxide. The direct dehydration of phenol over aluminum chloride or zinc chloride at 350°C. gives only a very small amount of diphenyl ether (191, 211). The yields can be substantially increased when thorium dioxide is used as a catalyst. Sabatier and Mailhe obtained a 50 per cent yield of diphenyl ether in this manner in the temperature range of 390–450°C. (235), while later workers were able to raise the yield to 64 per cent (37). The reaction is reversible, and the optimum temperature is 450°C. (37).

Diphenyl ether is obtained among other products from the destructive distillation of calcium phenyl salicylate (136). It is formed in good yield (40–50 per cent) when a mixture of sodium benzenesulfonate and potassium phenoxide is distilled (204).

The best laboratory method for the preparation of diphenyl ether is due to Ullmann and Stein (284), who discovered in 1905 that copper (301) catalyzes the reaction between alkali phenoxides and aryl halides. The wide applicability of this method has made possible the synthesis of the majority of the compounds described in the following.

Diphenyl ether is available in commercial quantities as a by-product of the manufacture of phenol by the hydrolysis of chlorobenzene with aqueous sodium hydroxide (18, 192) or sodium carbonate (107) under pressure at elevated temperatures (Dow Process). The reaction is catalyzed by copper (107). The formation of diphenyl ether by this process is reversible (105, 105a) and can be restrained by adding the ether to the reaction mixture (106, 108).

The use of diphenyl ether as a heat-transfer medium is due to its extraordinary heat stability. A eutectic mixture of 73.5 per cent of diphenyl ether and 26.5 per cent of diphenyl (Dowtherm A) (290) and a eutectic mixture of 85 per cent of diphenyl ether and 15 per cent of naphthalene (Dowtherm B) are recommended for this purpose in the temperature range of 230–400°C. (119).

Pyrolysis of diphenyl ether at red heat causes mainly dehydrogenation to dibenzofuran (97, 193, 198). The by-products are benzene, phenol, carbon monoxide, carbon dioxide, hydrogen, methane, and ethane (198). When the pyrolysis is carried out in an autoclave at 500–550°C. (60 atm.), 65 per cent remains unchanged after 5 hr. The remainder of the reaction mixture consists of resin, gaseous material, some phenol, and a trace of benzene (133). Pyrolysis at 500°C. under 100 atm. of hydrogen yields benzene, phenol, and water (206).

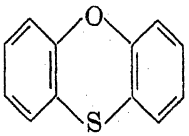
Under moderate conditions the hydrogenation of diphenyl ether in the presence of nickel catalysts gives some dicyclohexyl ether (14–42 per cent) (2). More drastic conditions (150–250°C.) favor the formation of cyclohexane and cyclohexanol (134, 152, 188, 206). At 500°C. (100 atm.) the main products are methane, cyclopentane, and methylcyclopentane (206). Cyclohexane and cyclohexanol are obtained as chief reduction products when reduced copper is used as a catalyst (80–150 atm., 200–220°C.) (153), whereas the hydrogenation over molybdenum sulfide at 350°C. gives benzene, cyclohexane, and phenol (194).

Diphenyl ether is unaffected by hydriodic acid at 250°C. (133) and by heating with zinc dust (126, 133). It is stable toward oxidizing agents such as chromium trioxide in acetic acid (126), but in the animal organism (rabbit) it is converted to 4-hydroxydiphenyl ether (257).

Diphenyl ether forms a crystalline complex $((C_6H_5)_2O \cdot AlX_3)_2$ with aluminum chloride and aluminum bromide (150).

The ether linkage is cleaved slowly by heating diphenyl ether with dilute but not with concentrated sodium hydroxide solution at 300°C. (192), with aqueous sodium carbonate (105a), or with aqueous sodium phenoxide solution under pressure (105), or by fusing with potassium hydroxide at 300°C. (4). The product in each case is phenol or a phenoxide. A 50 per cent yield of phenol is obtained in $4\frac{1}{2}$ days by treating diphenyl ether with sodium in liquid ammonia (249). The reaction is quantitative when 2 atom-equivalents of sodium are added to 1 mole-equivalent of the ether in liquid ammonia (240). Cleavage can also be effected by boiling 1 mole of diphenyl ether with 2–3 atom-equivalents of sodium, potassium, or lithium in 4–6 moles of dry pyridine in a current of nitrogen (212), or by shaking the ether with sodium potassium alloy in benzene solution at room temperature (196). Heating with dimethyl sulfate at 155–160°C. gives a 40.5 per cent yield of methoxybenzenesulfonic acid and a 26 per cent yield of methyl methoxybenzenesulfonate (22). At higher temperatures sulfonation rather than cleavage becomes the main reaction. Ethylmagnesium bromide at 170–190°C. converts diphenyl ether to phenol and 2-hydroxybiphenyl (253). Some 2-hydroxybiphenyl is also obtained on cleavage of the ether with sodium acetylide (85).

The ether oxygen is replaced with sulfur when diphenyl ether is heated with sulfur for 15 hr. at 350°C. (156). In the presence of aluminum chloride at 100°C. diphenyl ether reacts with sulfur to give phenothioxin (IV) (261, 262).



IV

Phenothioxin

IV. ALKYLDIPHENYL ETHERS

The three symmetrical ditolyl ethers have been prepared by dehydration of the corresponding cresols over thorium dioxide at 400–450°C. (235). With the exception of the *o*-isomer the cresols give good yields of dimethyldiphenyl ethers. Higher temperatures favor dehydrogenation to dibenzofurans. The method has been applied to more complex compounds (237, 238) and even to mixed ethers (236). In the latter case the reaction products consist of mixtures which have to be separated.

Buch obtained 4,4′-dimethyldiphenyl ether by the dehydration of *p*-cresol with zinc chloride at 300°C. (41). Some *m*-cresyl ether was formed when *m*-cresol was dehydrated with 10 per cent of aluminum chloride (211).

Pyrolysis of the aluminum cresoxides gives fairly good yields of symmetrical dimethyldiphenyl ethers (55, 89, 90). More general are the method of Nollau and Daniels (204), which consists in heating a sodium arylsulfonate with a potassium aryloxide, and the Ullmann (285) method, the latter usually giving the best yields.

The direct alkylation of diphenyl ether with one equivalent of alkyl chloride in the presence of aluminum chloride (52) gives chiefly mixtures of mono-, di-, and tri-alkyldiphenyl ethers at 100–150°C. Aliphatic 1,1-dihalides condense with diphenyl ether under similar conditions to give bis(phenoxyphenylalkyl)-diphenyl ethers (51). A di(*s*-hexyl)diphenyl ether has been obtained by the alkylation of diphenyl ether with 3-hexene and hydrofluoric acid (45).

Methyldiphenyl ethers frequently serve for the preparation of acids into which they can be converted by oxidation. Higher alkylated diphenyl ethers have been suggested as dielectric agents for transformers and as plasticizers for resins (52).

On cleavage with sodium in liquid ammonia 2-methyldiphenyl ether gives nearly equal amounts of phenol and *o*-cresol, while 4-methyldiphenyl ether yields *p*-cresol as the main cleavage product and a small amount of phenol under the same conditions (240). The behavior of the 3-methyl isomer is intermediate between that of the *o*- and the *p*-methyl compounds. The order of increasing effectiveness of the three groups in strengthening the linkage between oxygen and the substituted phenyl group against cleavage is *o*-CH_3, *m*-CH_3, *p*-CH_3 (157). In 2,4′-dimethyldiphenyl ether the main product of the reaction with sodium in liquid ammonia is *p*-cresol (61 per cent), as would be predicted on the basis of the foregoing results (240). 4-*tert*-Butyl-4′-methyldiphenyl ether gives a slightly higher yield (52 per cent) of *p*-*tert*-butylphenol and a 48 per cent yield of *p*-cresol on cleavage, showing that the *tert*-butyl group has a slightly greater effect on the carbon–oxygen bond than the methyl group (300).

V. ARYLDIPHENYL ETHERS

Phenyl-substituted ethers (aryloxydi- and ter-phenyls) have been obtained by the Ullmann reaction (179), by rearrangement of diphenyl ether with phenylsodium (179), by treating a Grignard reagent of diphenyl ether with silver bromide (62), and as by-products in the preparation of phenylphenols (38).

The naphthyl ethers are best prepared by the dehydration of the naphthols with dilute sulfuric acid (94, 95), zinc chloride (191), or anhydrous hydrogen chloride (191). This method is superior to the Ullmann method, particularly for the α-isomer (285). It is also used for the preparation of the phenanthryl ether from 9-phenanthrol (135). β-Naphthyl ether is obtained from the pyrolysis of aluminum β-naphthoxide (89), and both naphthyl ethers have been reported as by-products in the hydrolysis of chloronaphthalenes (38).

Naphthyl phenyl ethers have been prepared by the action of phenol upon the two naphthyldiazonium salts (129), and by the Ullmann reaction (285). β-Naphthol reacts with potassium nitroso-*p*-tolylsulfamate to give 30–40 per cent yields of β-naphthyl *p*-cresyl ether (208).

The phenyl (82, 279), methoxyphenyl (141), and naphthyl ethers (159) of anthraquinones are prepared by the sulfonate or Ullmann methods. 2-Phenoxyfluorene has been obtained in 18 per cent yield by heating 2-bromofluorene with potassium phenoxide and copper catalyst (176).

VI. PHENOXYQUINOLINES AND ISOQUINOLINES

The Ullmann reaction is used exclusively for the preparation of these ethers. 6-Phenoxyquinoline, 6-phenoxytetrahydroquinoline, and *N*-methyl-6-phenoxytetrahydroquinoline have been described as starting materials for a series of amines of therapeutic value (215). 6-Phenoxy-8-nitroquinoline and 6-phenoxy-8-aminoquinoline are the intermediates for the preparation of these amines (215). 5-Phenoxyisoquinoline has been described by Andersag (3).

VII. HALOGEN COMPOUNDS

The direct halogenation of diphenyl ether can be conducted in such a way that good yields of 4-halogenodiphenyl ethers are obtained. Chlorination is carried out in acetic acid (35) or in carbon tetrachloride at room temperature (186), bromination in carbon tetrachloride (258) or carbon disulfide in the presence of some iodine (186) or in acetic acid (34), and iodination with iodine monochloride in acetic acid (36). The 4,4′-dihalodiphenyl ethers are usually obtained in small amounts as by-products. A mixture of mono- and di-chlorodiphenyl ethers has been obtained by the chlorination of diphenyl ether with sulfuryl chloride (210). Halogenation of diphenyl ether with two molar equivalents of halogen or halogenation of the 4-halides gives good yields of the 4,4′-dihalogen compounds. The 4,4′-dichloro ether is accompanied by the 3,4-dichloro compound (35), whereas the 4,4′-dibromo compound is obtained free from isomers by the bromination of diphenyl ether in alcohol (291) and serves as a solid derivative for the identification of diphenyl ether. 4,4′-Diiododiphenyl ether can be prepared by iodination of the 4-iodo compound with iodine monochloride in acetic acid (36).

3,4,4′-Trichlorodiphenyl ether is produced in the chlorination of diphenyl ether, 4-chlorodiphenyl ether, and 3,4- and 4,4′-dichlorodiphenyl ethers (35).

A tetrabromodiphenyl ether of unknown orientation has been described by Cook (56). Halogenation with chlorine or bromine in the presence of aluminum chloride can be made to give diphenyl ethers containing more than four and less than ten halogens (39). With excess bromine and aluminum bromide, diphenyl ether can be exhaustively brominated. The resulting decabromo compound melts at 293°C. (26).

Monochloro, dichloro, monobromo, and dibromo derivatives of the three simple cresyl ethers have been described by Mailhe and Murat (187). Cook prepared both dibromo- (55) and tetrabromo-*m*-tolyl ethers (56), but the structures of the halogen tolyl ethers, all of which were obtained by direct halogenation, have not been determined.

Halogens can also be introduced into diphenyl ether by the diazotization of aminodiphenyl ethers and replacement of the diazonium group with chlorine (35), bromine (171), or iodine (36), or, in the case of bromine, by replacement of sodium sulfonate groups by direct bromination (259).

Halogenated phenols can be used in the Ullmann reaction for the preparation of halogenated diphenyl ethers if an excess of the phenol is used (228). Hydroxydiphenyl ethers are formed as by-products. The method works particularly well when the halogen in the aryl halide molecule is activated (242). The Ullmann reaction between dihalogenated benzenes and phenoxides can be limited to one of the halogens to give halogenated diphenyl ethers if two different halogens are present in the dihalogen derivative. 4-Chlorodiphenyl ether is obtained in this way by the reaction of *p*-chlorobromobenzene and potassium phenoxide (260). Some 4-phenoxydiphenyl ether is obtained as a by-product. The method has been used successfully for the preparation of a fluorodiphenyl ether from *m*-bromofluorobenzene (246).

Bromo- and iodo-diphenyl ethers have been prepared from acetoxymercuridiphenyl ethers by the action of bromine or iodine monochloride, respectively (247).

According to Rittler the halogenated diphenyl ethers are hydrolyzed to hydroxydiphenyl ethers by heating with aqueous alkali and copper catalyst under pressure (226). Heating with copper bronze at 200–220°C. converts 2-iododiphenyl ether to 2,2′-diphenoxydiphenyl (164). The bromodiphenyl ethers are used as halide components in the Ullmann reaction for the preparation of phenoxydiphenyl ethers (255).

The iododiphenyl ethers form Grignard reagents readily, the bromo compounds more slowly (66). The resulting reagents give the expected alcohols with carbonyl compounds (49, 66). Bromo- and iodo-diphenyl ethers react with alkyllithium by interconversion under mild conditions. The halogen may be ortho, meta, or para to the ether linkage (158).

The alkylation of halogenated diphenyl ethers with alkyl halides and aluminum chloride takes place much less readily than the corresponding alkylation of diphenyl ether. The products are predominantly monoalkyl derivatives (50).

In substitution reactions of halogenated diphenyl ethers the directive influence of the phenoxy groups largely determines the position of the new substituent (35).

VIII. NITRODIPHENYL ETHERS

The direct nitration of diphenyl ether with a slight excess of fuming nitric acid in a mixture of acetic acid and acetic anhydride at 25–30°C. gives a mixture of 2- and 4-nitrodiphenyl ethers and a small amount of dinitrodiphenyl ether (258). 4-Nitrodiphenyl ether can be partially frozen out of this mixture. When a large excess of fuming nitric acid is used under the same conditions, the product consists mostly of dinitrodiphenyl ether (258). Cleavage and oxidation account for the formation of oxalic and picric acid as by-products (174). The nitration of diphenyl ether with potassium nitrate and concentrated sulfuric acid at 70°C. gives an 80 per cent yield of 2,4,2′,4′-tetranitrodiphenyl ether (189).

4,4′-Dimethyldiphenyl ether upon nitration undergoes considerable cleavage to 2-nitro- and 2,6-dinitro-*p*-cresols (223). Depending on the nitration conditions, the 2-nitro-, 2,2′-dinitro-, and 2,6,2′,6′-tetranitro-*p*-cresyl ethers are also obtained. The nitration of 2-nitro-*p*-cresyl ether gives a mixture of dinitro-*p*-cresol and 2,2′-dinitro-*p*-cresyl ether (220). 4-Methyldiphenyl ether is intermediate in stability between *p*-cresyl ether and diphenyl ether. It yields nitrophenols and cresols, owing to nitrative cleavage, and a single dinitrodiphenyl ether which probably has the nitro groups in the 2- and 4′-positions (224).

The nitrodiphenyl ethers are usually prepared from *o*- and *p*-nitrochlorobenzenes (32a), *p*-nitrofluorobenzene (219), *m*-nitrobromobenzene (285), 2,4-dinitrochlorobenzene (28), picryl chloride (302, 304), and the appropriate phenoxides. With the more highly activated halides the reaction takes place readily without a catalyst. The 2,4-dinitrodiphenyl ethers form rapidly when an alcoholic solution of 2,4-dinitrochlorobenzene is added to an aqueous alkaline solution of the phenol, and the mixture is refluxed on a steam bath. They are used for the identification of phenols (28).

Copper catalyst is usually required for the preparation of 3- and 4-nitrodiphenyl ethers from *m*-bromo- and *p*-chloro-nitrobenzenes (32a), whereas it is said to be neither helpful nor desirable in the preparation of 2-nitrodiphenyl ethers from *o*-chloronitrobenzene (120). Nitrophenols, which react only difficultly with *p*-chloronitrobenzene, give good yields of diphenyl ethers with *p*-fluoronitrobenzene (219). Both *o*- and *p*-nitrophenols react with 2,4-dinitrochlorobenzene (217) and picryl chloride without a catalyst (304).

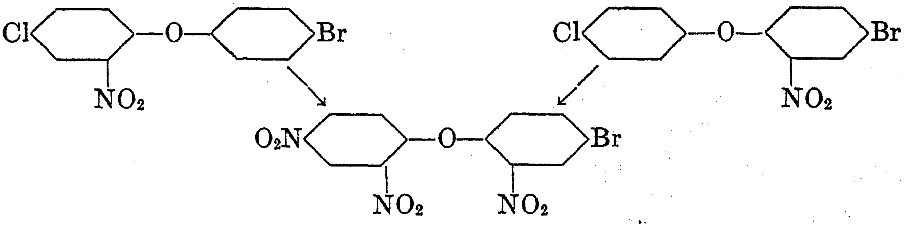
Nitrodiphenyl ethers can be prepared in excellent yield by the reaction of *p*-dinitrobenzene with phenoxides (174). Heating *o*- or *p*-dinitrobenzene with dry potassium cyanide gives 2,2′- or 4,4′-dinitrodiphenyl ether (173).

The nitro group in nitrodiphenyl ethers can be reduced in good yield to amino groups by means of stannous chloride (174), tin and hydrochloric acid (57), iron and acetic acid (99, 185), iron and ferric chloride (64), iron and hydrochloric acid (258), alkaline ferrous hydroxide (182), zinc and calcium chloride (258), ammonium sulfide (61, 183), or by catalytic reduction (36, 263). The electrolytic reduction in alcoholic alkali converts nitrodiphenyl ethers to azoxy compounds which separate out, owing to their low solubility (103). In acid solution the electrolytic reduction of the nitro compounds yields amines. Hydrazo compounds result from the reduction of nitrodiphenyl ethers with zinc dust and alcoholic alkali (104). The corresponding azo compounds can be obtained by oxidation with air or ferric chloride.

Nitro groups ortho or para to the oxide linkage weaken this linkage, thus allowing cleavage by sodium hydroxide (184, 189, 303), particularly in alcoholic solution (223), and by nitrogen bases. The latter case has been thoroughly investigated by a number of different workers. 2,4-Dinitrodiphenyl ether is cleaved to a small extent by aqueous alcoholic ammonia at 40–50°C. (27). When heated with aniline the 2,4-dinitrodiphenyl ethers give good yields of 2,4-dinitrodiphenylamine (27, 121, 203). If hydrazine is used instead, the cleavage is complete in a few minutes and gives a good yield of a benzotriazole and a phenol (27). Phenylhydrazine reacts more slowly, with the formation of 2-phenyl-2-benzotriazoles (27). A single nitro group in the 2-position of diphenyl ether activates the ether linkage sufficiently for cleavage by hydrazine but not by phenylhydrazine (27). The reaction with piperidine is most commonly applied for establishing the structures of nitrodiphenyl ethers. Cleavage takes place very slowly at 100°C. when one 2- or 4-nitro group is present but can be carried to completion in a few minutes with 2,4-dinitrodiphenyl ethers (43, 161). In all the cleavage reactions the amine nitrogen becomes attached to the more heavily nitrated ring and the second ring is isolated as a phenol (81, 161).

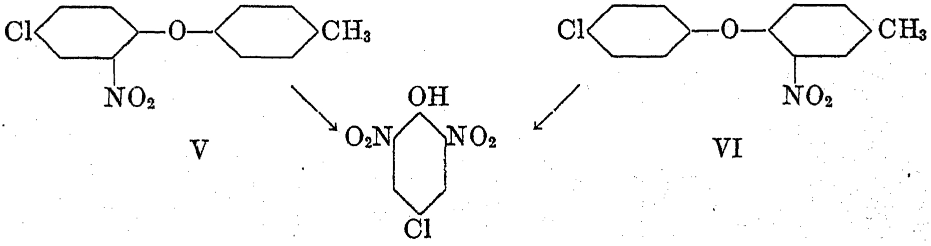
Nitrohalogenodiphenyl ethers can be prepared by nitrating halogenated diphenyl ethers. The phenoxy group orients the nitro groups to the 4-position when possible. When both 4-positions are blocked, the compound is usually nitrated in the 2-position. Two nitro groups in the 2- and 4-position of one ring inhibit further nitration in that ring (81).

In a few special cases chlorine and bromine are eliminated during nitration, as in the following examples (80),



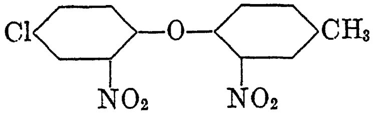
and in the nitration of 4-nitro-4′-bromodiphenyl ether, which gives 2,4,2′,4′-tetranitrodiphenyl ether (218). Iodine is more frequently eliminated (36).

Occasionally nitrative cleavage is also observed. The two nitro-4′-chloro-4-methyldiphenyl ethers (V and VI) react with cold fuming nitric acid to give 2,6-dinitro-4-chlorophenol as the main product.


V

VI

Yet the dinitro ether (VII) which is obtained by the nitration of V and VI under mild conditions (fuming nitric acid diluted with acetic acid) cannot be cleaved by cold fuming nitric acid (80). When VII is heated with fuming nitric acid, partial replacement of chlorine by a nitro group occurs.



VII

The halogenation of nitrodiphenyl ethers is considerably more limited in scope, since halogens cannot ordinarily be introduced into the nitrated rings. Raiford and coworkers were unable to introduce bromine into 2,2′,4-trinitro-, 2,3′,4-trinitro-, and 2-nitro-4′-bromo-diphenyl ethers (217).

In nitrodiphenyl ethers which contain a non-nitrated ring, second or third substituents, such as halogens, nitro, or other groups, tend to enter this ring. The mononitrodiphenyl ethers are first halogenated in the 4′-position (181, 242). Forced halogenation introduces a second halogen in the 2′-position (242). Under more rigorous conditions 2-nitrodiphenyl ether can be converted to 2-nitro-2,4,4′-tribromodiphenyl ether (180).

IX. HYDROXYDIPHENYL ETHERS

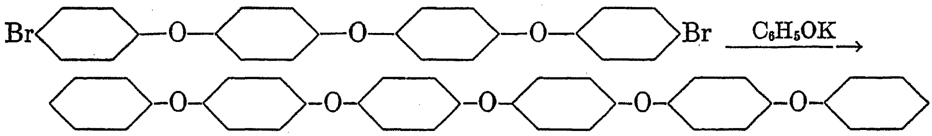
The Ullmann reaction can be used to prepare hydroxydiphenyl ethers directly from halogenated phenols and phenoxides (91, 147, 174, 213). Yields of 20–30 per cent may be obtained by the use of a large excess of phenoxide. In most cases the methyl ethers of the halogenated phenols are used instead, because of the higher yields of methoxydiphenyl ethers (286). Demethylation to the desired hydroxydiphenyl ethers is effected by heating with aluminum chloride in benzene (286), hydrobromic acid under pressure (269), hydriodic acid in acetic acid (174), or potassium hydroxide in ethylene glycol (293).

Small yields of hydroxydiphenyl ethers result when diazonium salts are decomposed with an excess of dihydric phenols such as catechol (205). 2-Hydroxydiphenyl ether has been isolated from the electrolytic oxidation of phenol (77), and 4,4′-dihydroxydiphenyl ether from the oxidation of phenol with chromyl chloride (71).

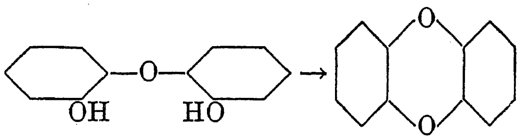
Hydroxydiphenyl ethers can be obtained from aminodiphenyl ethers through the diazonium reaction (101, 146, 174), and from chloro- or bromo-diphenyl ethers by high-pressure hydrolysis (226). A trinuclear hydroxydiphenyl ether has been described by Goldschmidt and coworkers as a product of the dehydrogenation of *o*-cresol with lead dioxide (91), but its structure has not been definitely established.

The three simple monohydroxydiphenyl ethers have been investigated most completely. Four dihydroxydiphenyl ethers and one simple trihydroxydiphenyl ether have also been described. The 2-, 3-, and 4-hydroxydiphenyl ethers have bactericidal properties (146, 147, 295), which can be further increased by halogenation or alkylation (295). 4-Hydroxydiphenyl ether has been suggested as an antioxidant (44).

The hydroxy groups in the foregoing compounds can be acylated (174), alkylated (123, 205), and allylated (295). The allyl ether of 4-hydroxydiphenyl ether has been rearranged to 3-allyl-4-hydroxydiphenyl ether by heating in diethylaniline (295). Aryl ethers of hydroxydiphenyl ethers may be prepared from the sodium salts of these compounds by heating with aryl halides and copper catalyst (102, 207). Staudinger and Staiger have built up chains of four, five, and six nuclei separated by oxygen by the reaction of *p*-dihalogen compounds with potassium phenoxide (255), e.g.:



2,2′-Dihydroxydiphenyl ether (VIII) is dehydrated to dibenzodioxin (IX) in good yield by heating with hydrobromic acid and a trace of red phosphorus at 190°C. (286). The dehydration may also be effected by heating with phosphorus oxychloride (286) or with zinc chloride (270).



VIII IX

Dibenzodioxin

The same product is obtained when 2,2′-dimethoxydiphenyl ether is heated with hydrobromic acid as above. The reaction is applicable to more complex ethers unless a third hydroxyl group occupies a position ortho or para to one of the hydroxyl groups involved in the cyclization (270). Tomita has reported the preparation of IX by the dehydrogenation of 2-hydroxydiphenyl ether with nitrobenzene and copper oxide (270).

Alkyl groups may be introduced into the phenolic ring of hydroxydiphenyl ethers by acylation and Clemmensen reduction of the resulting acylphenols or by hydrogenation of an allylphenol (295). Other alkylated hydroxydiphenyl ethers have been prepared through the Ullmann reaction with alkylphenoxides and alkylated aryl halides (213).

Chlorine or bromine may be introduced ortho or para to a hydroxyl group by chlorination with sodium hypochlorite or by direct bromination with bromine in acetic acid (295). Böhmer obtained a 13 per cent yield of 4′-bromo-4-hydroxydiphenyl ether by heating *p*-hydroxybenzenediazonium sulfate with hydrobromic acid (25). More commonly such halogen compounds are prepared by the halogenation of methoxydiphenyl ethers (171), or from aminomethoxydiphenyl ethers by diazotization, replacement, and demethylation (139).

4-Methoxydiphenyl ether is brominated in the phenoxy group more rapidly than 4-nitro, 4-bromo-, and 4-methyl-diphenyl ethers and even diphenyl ether itself. These data constitute part of the evidence that the tautomeric or inductive effects of the substituent may be transmitted across the ether linkage from one ring to the other (34). In the halogenation of 4-methoxydiphenyl ether the halogen enters the 4′-position (32, 207). If this position is blocked the halogens go into the 3-position, unless the blocking group is more strongly directing than the methoxy group (32). 2-Methoxydiphenyl ether yields a mixture of 5-bromo-2-methoxydiphenyl ether and 4′,5-dibromo-2-methoxydiphenyl ether on bromination (171) but only 4′-iodo-2-methoxydiphenyl ether on iodination (32).

2′-Bromo-2-hydroxydiphenyl ether is dehydrohalogenated to dibenzodioxin by heating with copper and copper acetate (139).

Many nitromethoxydiphenyl ethers have been described, presumably because they are easily obtained by the condensation of nitrohalobenzenes with the alkali salts of methoxyphenols (29, 42, 207, 244). A few have been prepared by the nitration of methoxydiphenyl ethers. The nitration of 2-methoxydiphenyl ether gives the 5-nitro compound as main product (80 per cent) and a small amount (20 per cent) of the 4-nitro isomer (170). The structure of the single nitration product of 4-methoxydiphenyl ether (160) has been established as 3-nitro-4-methoxydiphenyl ether (32), but no data are available on the halogenation and nitration of 3-methoxydiphenyl ether.

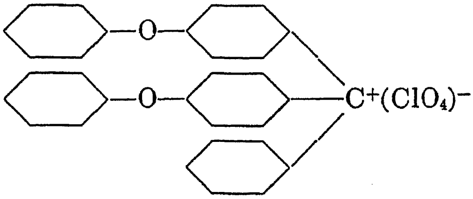
The results of the substitution reactions allow the conclusion that the methoxy group in the 2- and 3-positions has a somewhat stronger orienting effect than the phenoxy group. This effect becomes even greater when the phenoxy group carries a nitro substituent (42, 244). Nitration and halogenation of 2′-nitro-, 4′-nitro-, and 2′,4′-dinitro-2-methoxydiphenyl ethers give the 5-substituted compounds first. The second substituent goes usually to the 4-position. An exception is the nitration of 2′,4′-dinitro-2-methoxydiphenyl ether, where the second nitro substituent occupies the 3-position so that apparently the phenoxy group has no effect whatsoever (244). The chlorination of this same compound yields the 4,5-dichloro derivative. In 4′-nitro-2-hydroxydiphenyl ether the first bromine substitutes in the 5-position, the second in the 4-position (42).

Methoxy groups in the para-position strengthen the bond between the ether oxygen and the substituted phenyl group toward cleavage with sodium in liquid ammonia, while meta and particularly ortho methoxy groups weaken this linkage (157). Thus 2,3′-dimethoxydiphenyl ether yields 76 per cent of *m*-methoxyphenol and 24 per cent of guaiacol, while 3,4′-dimethoxydiphenyl ether is cleaved to give 92 per cent of *p*-methoxyphenol and 8 per cent of *m*-methoxyphenol (157).

X. ALCOHOLS

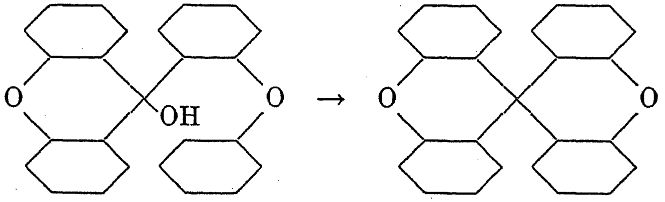
All known alcohols derived from diphenyl ether contain the hydroxyl group on a carbon directly connected to one of the rings. They have been prepared from hydroxydiphenyl ether by condensation with formaldehyde (295), from aldehydes by the Cannizzaro reaction (175), from ketones by reduction with aluminum isopropoxide (46) or by the Grignard reaction (49, 65, 66, 196), and from esters by the addition of Grignard reagents (280).

A few of these compounds are solid. Most of them are obtained in the form of oils which are difficult to purify. Dilthey and coworkers were able to isolate alcohols of the triarylcarbinol type in the form of colored crystalline carbonium salts such as X (66):

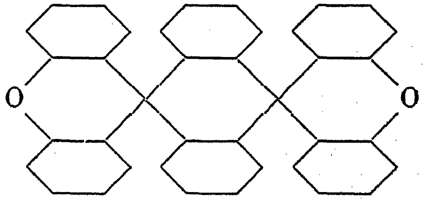


X

The reaction of the Grignard reagent from 2-iododiphenyl ether with xanthones or anthraquinone leads to alcohols which are readily cyclized to beautifully crystalline spiro compounds (XI and XII), which are characterized by high melting points and very low solubilities (49).

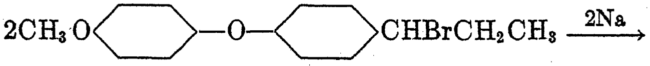


XI

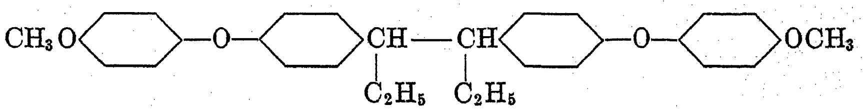


XII

The secondary (46) and tertiary alcohols (196) are readily converted to halides. The bromide (XIII) reacts with sodium to give an analog of hexestrol (XIV) (46).



XIII



XIV

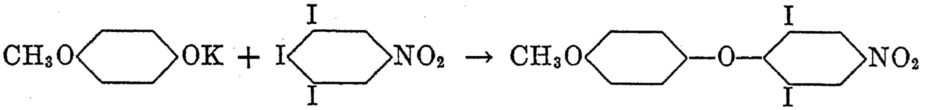
XI. ALDEHYDES

In 1898 Gattermann obtained 4-phenoxybenzaldehyde in approximately 50 per cent yield by the method which bears his name (84). According to Slotta and Soremba (250), the yield in this reaction can be raised to 70–80 per cent. The modification of Adams and Montgomery (1) gives a 50 per cent yield of the same compound. Lock and Kempter (175) prepared the three monoaldehydes by the Rosenmund reduction of the acid chlorides. The Etard reaction was unsuitable for the 3-isomer, since the intermediate double salt could not be decomposed (175).

Nitro aldehydes of diphenyl ether are obtained in good yield by condensing *p*-nitrofluorobenzene with the potassium salts of hydroxybenzaldehydes (219). The nitrochlorobenzenes (ortho or para) may be used for the same purpose. The reaction is carried out by adding the salt of the hydroxy aldehyde in small amounts to the hot halogen compound (36, 263).

The synthesis of the methoxy aldehydes is difficult. The Gattermann reaction is tedious and unsatisfactory (118), although it has been used for the preparation of various methoxy aldehydes (293). An attempt to use the *N*-methylformanilide method has met with failure (294), and the Etard reaction is impractical because of low yields (246). An example of the Ullmann reaction is reported by Robinson and Sugasawa (233), who condensed isovanillin with 3-bromo-4-methoxybenzaldehyde in pyridine in the presence of potassium carbonate and copper bronze. The method of McFayden and Stevens appears most promising for the synthesis of the methoxy aldehydes of diphenyl ether (118, 292).

The diiodomethoxy aldehyde required for the thyroxine synthesis is usually prepared from triiodonitrobenzene, which possesses sufficiently reactive halogen for the condensation with the monomethyl ether of hydroquinone under mild conditions.



XV

The resulting nitro compound (XV) is reduced to the amine, which is diazotized and treated with cuprous cyanide. The aldehyde is obtained by the Stephen reduction of the resulting nitrile (115).

4-Phenoxybenzaldehyde can be nitrated in the 4′-position by cautious nitration with concentrated nitric acid at 15–20°C. The 2′-nitro compound is obtained as a by-product. When 4-phenoxybenzaldehyde is nitrated with a mixture of nitric acid and sulfuric acid below 5°C., the 2,2′,4′-trinitroaldehyde is produced (250). An iodine atom can be introduced into the 4′-position of 4-phenoxybenzaldehyde by iodination with iodine monochloride and iodic acid (250).

The aldehyde group in aldehyde derivatives of diphenyl ether can be reduced by the Clemmensen method (154) and oxidized with potassium permanganate (84), sodium dichromate and sulfuric acid (263), or silver oxide (175). These aldehydes form the usual derivatives, such as oximes, anils, phenylhydrazones (84, 175), and semicarbazones (118). They can be condensed with nitromethane (251), although sometimes in poor yield (256), with hydantoin (112, 251), glycine anhydride (112), acetylglycine (48), and hippuric acid (116, 251), and they undergo the Perkin reaction (112, 175). The products of these condensation reactions are intermediates in the preparation of β-arylethylamines (251, 256), α-amino acids (112), α-keto acids (48), and α-arylacetic acids (155).

Methoxy aldehydes can be demethylated with aluminum chloride. A side reaction sometimes causes the elimination of the formyl group as carbon monoxide. This reaction is dependent upon the relative positions of the groups involved (293).

XII. KETONES

4-Acyl- or 4-aroyl-diphenyl ethers are obtained in good yield from diphenyl ether through the Friedel–Crafts reaction (145). The acylating agents may be acid chlorides (145), anhydrides (21, 46, 145), or aryl esters (60). By using an excess of acyl halide (2.5–3 moles) and aluminum chloride (2 moles), two acyl groups can be introduced into diphenyl ether by this method. The second group enters the 4′-position (65). 4-Nitrodiphenyl ether undergoes the Friedel–Crafts reaction in the para-position of the non-nitrated ring (65, 263). When both para-positions are occupied by methyl groups, acetylation and benzoylation occur in the 2-position (221, 222). Benzoic and succinic anhydrides react with diphenyl ether and aluminum chloride with formation of *o*-(*p*-phenoxybenzoyl)benzoic acid and *o*-(*p*-phenoxybenzoyl)propionic acid (145). Cinnamoyl chloride reacts slowly under similar conditions but gives an excellent yield of 4-cinnamoyldiphenyl ether (151).

4-Alkoxydiphenyl ethers are acylated first in the 4′-position (66), and then ortho to the alkoxy group (272). 2-Alkoxydiphenyl ether undergoes the Friedel–Crafts acylation to give 4′,5-diacyl compounds (267, 268). When each ring contains one methoxy group, the methoxy groups determine the positions of the entering acyl groups. Thus, 2,2′-dimethoxydiphenyl ether yields 5,5′-diacyl compounds (267), and 4,4′-dimethoxydiphenyl ether gives 3,3′-diacyl derivatives (272). The methoxy or alkoxy groups may be retained in these reactions or cleaved to hydroxyl groups, depending on the reaction conditions (272).

4-Methoxydiphenyl ether was found to react with phosgene and aluminum chloride to give a good yield of 4,4′-di(4-methoxyphenoxy)benzophenone (66). Oxalyl chloride can be used in the place of phosgene in this case (15). Diphenyl ether and 4-methyldiphenyl ether are converted to substituted benzils by reaction with oxalyl chloride and aluminum chloride. 3-Methyl-, 3,3′-dimethyl-, 3-methoxy-, 3,4-dimethoxy-, and 3,4′-dimethoxydiphenyl ethers, on the other hand, yield xanthones with these reagents. 4,4′-Dimethoxy- and 4,4′-dinitro-diphenyl ethers do not react under the same conditions (15).

Acyl- and aroyl-diphenyl ethers can also be obtained in good yield by the

Ullmann reaction. The starting materials are halogenated aromatic ketones and phenols or hydroxyphenyl ketones and aryl halides (65, 66, 75).

Acetyldiphenyl ethers have been prepared from the corresponding acid chlorides of diphenyl ether by the acylation of ethyl sodioacetoacetate with the acid chloride, and hydrolysis of the resulting ester (294). Houben reports a 70 per cent yield of 4-trichloroacetyldiphenyl ether by the application of his ketone synthesis to diphenyl ether (130).

For proof of structure or for preparative purposes, acetyl- and chloroacetyldiphenyl ethers may be converted to acids by oxidation with alkaline permanganate (75, 245) or hypohalites (294). The Clemmensen reduction of the ketones yields alkyldiphenyl ethers (273), and the Meerwein reduction leads to the corresponding secondary alcohols (46).

Methyl ketones derived from diphenyl ethers can be converted to chalcones in good yield (65, 67). Some of the simpler ketones and particularly the chalcones form colored complex salts with sulfuric acid, stannic chloride, and perchloric acid (65).

Chloroacetyldiphenyl ethers have been used as intermediates in the preparation of amino alcohols of therapeutic importance (271).

XIII. ACIDS

4-Phenoxybenzoic acid was prepared by Klepl in 1883 by the hydrolysis of its phenyl ester, which was one of the pyrolysis products of *p*-hydroxybenzoic acid (148). The 2-phenoxy isomer was first obtained by Graebe in 1888 by heating the sodium salt of phenyl salicylate at 280–300°C. (96). The yield in this rearrangement was 25–30 per cent. Griess prepared all three isomers by decomposition of the diazonium salts of the three aminobenzoic acids with phenol (98) but reported no yields, and this method is not recommended for preparative purposes.

In 1904 Ullmann announced his copper-catalyzed reaction for the preparation of 2-phenoxybenzoic acid (278), and Fosse described the preparation of the same substance by the rearrangement of phenyl carbonate (78). The latter compound is stable to heating at 300°C. when pure, but rearranges with evolution of carbon dioxide and phenol when heated in the presence of sodium or potassium carbonate. Small amounts of carbonate give phenyl 2-phenoxybenzoate, while an excess of carbonate causes the formation of the free acid. Since salol gives the same products in the same ratio when pyrolyzed under these conditions, it has been suggested as an intermediate in the reaction (78). A recent patent (209) covers the preparation of phenyl 2-phenoxybenzoate by the pyrolysis of purified phenyl carbonate (from phosgene and sodium phenoxide) in the presence of 1 per cent of potassium carbonate at 230–270°C. The phenyl ester is hydrolyzed in good yield by heating with water and small amounts of sulfuric or sulfonic acids (149).

The Ullmann reaction involves heating the sodium or potassium salt of an *o*- or *p*-chlorobenzoic acid with a phenoxide and copper catalyst. With slight modifications the original procedure (278) is applicable to the preparation of larger amounts of acids in good yield (36). The method has been used for the preparation of nitro acids (281), chloro acids (92, 287, 288), and methoxy acids of diphenyl ether (214, 282, 287, 289). The methoxy group may be located in either ring, while nitro groups are preferentially placed in such positions of the halogenated benzoic acids that they further activate the halogen. In dihalogenated benzoic acids the ortho halogen reacts in preference to the meta and para halogens, and the reaction is easily limited to one halogen.

Hydroxybenzoic acids are rarely used as phenolic components in the Ullmann reaction. An example of this kind is described by Haeussermann and Bauer (100). The esters of hydroxybenzoic acids, on the other hand, usually give good yields of diphenyl ethers when heated with aryl halides and copper catalyst (76, 118, 292, 294). When more complex esters are used the yields are decreased, owing to a number of side reactions. The most important of these is the replacement of halogen by hydrogen in the aryl halide molecule, a result which has been observed with bromomethoxybenzoic esters (75, 143), bromoalkoxyphthalic esters (142), and iodomethoxyphthalic esters (144), and may actually become the main reaction (75). Similar dehalogenations have also been described in the Ullmann reaction of 2-bromofluorene (176) and of 1,2,3,5-tetrabromobenzene with potassium phenoxide (285).

Another side reaction particularly pronounced in the phthalates or esters of other polycarboxylic acids consists in the alkylation by the ester of the free phenolic group required for the Ullmann reaction (142). This reaction can be used for alkylating phenols in a yield of 70–85 per cent. In its more general aspect it consists in heating the potassium salts of phenols with molecular proportions of alkyl phthalates at 190–200°C. (144). Methyl benzoate can be used as an alkylating agent in this manner, but the yield of phenol ether is much lower. Since the accumulation of carbomethoxy groups is largely responsible for this latter side reaction, Faltis and coworkers (75) suggest the synthesis of diphenyl ethers with methyl and (or) acetyl groups which can be converted to carboxyl groups after the diphenyl ether is formed. This indirect method has been widely used for the preparation of numerous acids.

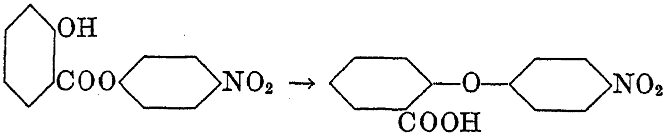
The methyl groups in diphenyl ethers can be oxidized by dichromate, acetic acid, and sulfuric acid at 44°C. (34). The complete decomposition of 4′-nitro-4-methyldiphenyl ether by this reagent, as reported by Cook (53), is ascribed to cleavage caused by too high concentration of oxidizing agent and sulfuric acid (34). Some cleavage, however, is encountered in the oxidation of *p*-tolyl ethers even under mild conditions. According to Brewster and Slocombe, this is due to the shift of electrons from the ether oxygen atom to the *p*-tolyl nucleus. If the second nucleus is nitrated in the 2- or 4-position, the opposite shift of electrons hinders the cleavage reaction and the yields of acids increase (34). Permanganate has been used with good success for the oxidation of methyl groups to carboxyl groups in the diphenyl ether series. The yield in this case seems to depend to a great extent on the solvent used in the oxidation (292).

Acids of diphenyl ether have been obtained from the carbonation of sodium derivatives (179), lithium derivatives (85, 86, 158), and Grignard reagents of diphenyl ethers (158).

Suter and Oberg prepared *p*-(4-nitrophenoxy)benzoic acid by oxidation of the corresponding aldehyde with sodium dichromate and sulfuric acid (263). This method is obviously limited to easily accessible aldehydes. The second method of Suter (258), which consists in introducing a cyano group by the Sandmeyer reaction and hydrolyzing the nitrile, is equally limited.

Hydroxy acids may be prepared by the demethylation of methoxy acids with hydriodic acid (292), hydrobromic acid (294), aluminum chloride (293), or alkali in high-boiling hydroxylic solvents (293). 3-Phenoxy-4-methoxybenzoic acid is resistant to hydriodic acid but can be demethylated with hydrobromic acid at 150°C. or with aluminum chloride (293). The demethylation of the methoxy acids with alkali may cause decarboxylation, depending upon the structure of the acids (293). Hydroxy acids may be obtained directly from hydroxydiphenyl ethers through the Kolbe reaction (225, 227). The carboxyl group enters a position ortho to each hydroxyl group in the original ether.

o-(*p*-Nitrophenoxy)benzoic acids (XVII) have been obtained by the rearrangement of *p*-nitrophenyl *o*-hydroxybenzoates (XVI) (274).

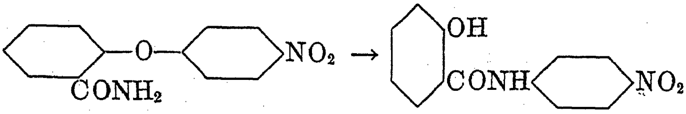


XVI XVII

The reaction gives a good yield (70 per cent) and takes place when the esters (XVI) are heated at 100°C. in a basic solution. It is inhibited by a nitro substituent para to the hydroxyl group in the esters (XVI). Salol requires much more drastic conditions for the analogous rearrangement (78).

Diphenyl ether acids with a carboxyl group in the 2-position are readily cyclized to xanthones by treating with phosphorus pentachloride and aluminum chloride (19) or by heating with concentrated sulfuric acid at 100°C. (5, 19, 70, 78, 79, 92, 96, 162, 281, 282, 283, 287, 289). Gottesmann (93) claims a quantitative yield of xanthones when the acids are heated for a few minutes with acetyl chloride and 1/40 of the amount of sulfuric acid.

The acids of diphenyl ethers are readily converted to acid halides, esters (263), and amides (6). The amide of *o*-(4-nitrophenoxy)benzoic acid (XVIII) rearranges when warmed with bases at 50°C. according to the following equation:



XVIII

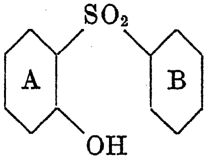
The anilide corresponding to XVIII is rearranged more easily than XVIII, and the amide of *o*-(2,4-dinitrophenoxy)benzoic acid is converted to the *N*-nitrophenylsalicylamide by heat alone (275).

The carboxyl group in the acids of diphenyl ether can be eliminated by heating with barium hydroxide (96, 148) or by the Reichstein method (144). Alkali fusion at 210–250°C. breaks the ether linkage and yields *o*- or *p*-hydroxybenzoic acid when the structure of the original acid permits (76, 112, 115). Sodium in liquid ammonia causes smooth cleavage of 2- and 4-phenoxybenzoic acids with the formation of phenol and benzoic acid or partially hydrogenated benzoic acid (240).

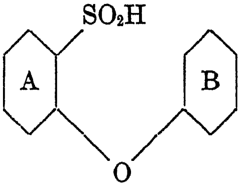
The nitration of 2-phenoxybenzoic acid gives a dinitro acid of unknown configuration, which undergoes nitrative cleavage when warmed with fuming sulfuric acid. The cleavage products are nitrosalicylic acid and 2,4-dinitrophenol (6). 2-Phenoxy-5-nitrobenzoic acid is nitrated and halogenated in the 4′-position (36).

XIV. SULFINIC, SULFONIC, AND SELENINIC ACIDS

p-Phenoxybenzenesulfinic acid was prepared by Suter by the reduction of the corresponding sulfonyl chloride with sodium sulfite and sodium carbonate. It was found to be quite unstable and could not be isolated in a pure state (259). Stable derivatives of *o*-phenoxybenzenesulfinic acid were synthesized by Krishna by the condensation of 2-chloro-5-nitrobenzenesulfinic acid with sodium phenoxides in boiling aqueous sodium hydroxide solution (157a). A number of *o*-phenoxybenzenesulfinic acids (XX) have been prepared by the rearrangement of *o*-hydroxysulfones (XIX) with alkali (140, 166, 167, 296, 298, 299).



XIX

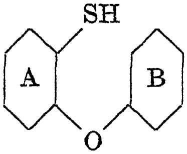


XX

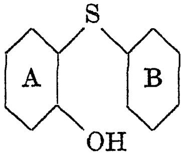
This reaction takes place at room temperature if a nitro group occupies a position ortho to the sulfone linkage in ring B. A *p*-nitro group in this ring permits the rearrangement, but it takes place more slowly. In the absence of nitro substituents the sulfones (XIX) are stable even when heated to 250°C. with alkali. A carboxyl group in the 2-position or a nitro group in the 3-position of B fails to promote the rearrangement (140). Sulfones in which B is 2,4-dinitrophenyl, and *p*-hydroxysulfones undergo cleavage rather than rearrangement with alkali (140, 166). Bis-1-(2-hydroxynaphthyl) sulfone is converted to the sulfinic acid by heating with alkali at 150°C. (298). The acid cannot be isolated, however, since it loses sulfur dioxide under these conditions. The structures of the sulfinic acids are usually determined by permanganate oxidation and removal of the sulfonic acid grouping by steam distillation. The sulfur-free products have been synthesized in several cases (167, 296a). Sometimes the methyl sulfinate is accessible to synthesis (299).

2-Phenoxybenzenesulfinic acids cyclize when they are treated in the cold with acetic anhydride containing a trace of sulfuric acid or with sulfuric acid. The products are mixtures of phenothioxins and their oxides (157a).

Sulfinic acids of diphenyl ether can be reduced to diphenyl ethers with zinc and acetic acid (296) and to phenoxythiophenols with zinc and hydrochloric acid (296). The *o*-phenoxythiophenols (XXI) rearrange under the influence of alkali to give *o*-hydroxydiphenyl sulfides (XXII) if they contain a nitro group in the ortho-position of ring B (140).



XXI



XXII

Oxidation of the sulfides (XXII) with hydrogen peroxide in acetic acid regenerates the hydroxysulfones (XIX) (167, 299).

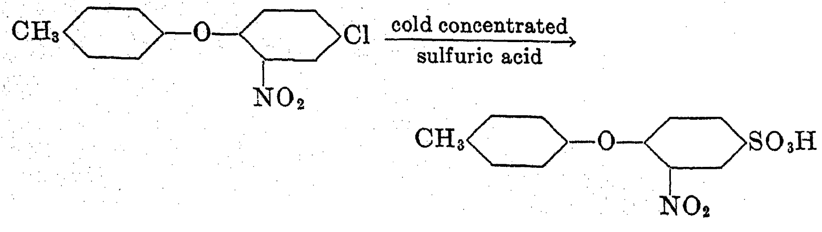
The direct sulfonation of diphenyl ether with 95 per cent sulfuric acid and acetic anhydride at 100°C. gives an excellent yield (93 per cent) of *p*-phenoxybenzenesulfonic acid (259), which can be isolated as the sodium salt. Its structure was established by conversion of the sodium sulfonate to 4,4′-dibromodiphenyl ether by direct bromination in water solution. The intermediate bromophenoxybenzenesulfonate can be isolated. Disulfonation takes place preferentially when diphenyl ether is sulfonated with concentrated sulfuric acid without a solvent at 100°C., because the monosulfonic acid which is first formed is soluble in concentrated sulfuric acid and is further sulfonated more rapidly than the almost insoluble diphenyl ether. The disulfonic acid is assigned the 4,4′-configuration, since its sodium salt yields 4,4′-dibromodiphenyl ether on bromination (259).

2-Nitro-, 2,4-dinitro-, and 2,4-dinitro-6-carboxydiphenyl ethers are sulfonated with concentrated sulfuric acid at temperatures at or above 100°C., presumably in the 4′-position (56, 138, 214). 4-Nitrodiphenyl ether requires 33 per cent fuming sulfuric acid for sulfonation (138). The position of the sulfonic acid group in these compounds has not been established. The structures of other sulfonic acids prepared by Cook and coworkers by sulfonating various diphenyl ethers are also unknown (53, 57, 58, 59).

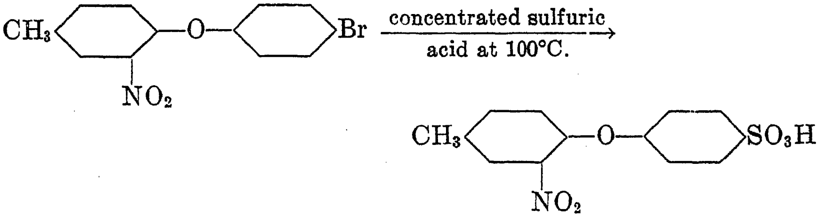
Chlorosulfonic acid acts on diphenyl ether at 25–30°C. with formation of the 4,4′-disulfonyl chloride (132, 259). The corresponding diamide is used as a solid derivative of diphenyl ether because of its sharp and characteristic melting point (132). 4,4′-Dibromodiphenyl ether is sulfonated in the 2- and 2′-positions with chlorosulfonic acid (261).

The sulfonation of diphenyl ether with aminosulfonic acid takes place when the mixture is heated to 160–170°C. The product is ammonium *p*-phenoxybenzenesulfonate (216).

The following unusual cases of sulfonation are described by Fox and Turner:

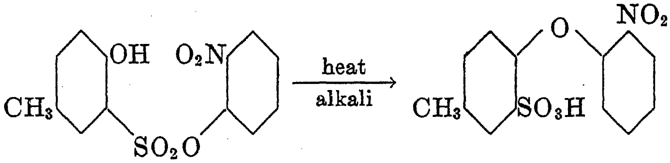


(80)



(81)

o-Nitrophenoxybenzenesulfonic acids (XXIV) can be prepared by the rearrangement of the *o*-nitrophenyl *o*-hydroxybenzenesulfonates (XXIII), a rearrangement which is analogous to that of the nitrophenyl salicylates (274).

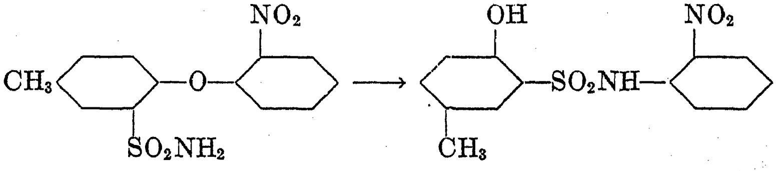


XXIII XXIV

Sodium phenoxybenzenesulfonates react with phosphorus pentachloride to give sulfonyl chlorides (259). This method constitutes the only good way to prepare 4-phenoxybenzenesulfonyl chloride, since chlorosulfonic acid causes disulfonation of diphenyl ether. 4-Phenoxybenzenesulfonyl chloride is brominated in the 4′-position, and the product is identical with the chlorosulfonation product of 4-bromodiphenyl ether. Amides and anilides of the sulfonic acids are readily prepared from the chlorides and serve as derivatives. Reduction of the sulfonyl chlorides with zinc and sulfuric acid or with stannous chloride, hydrochloric acid, and acetic acid gives thiophenols (259, 264), which can be converted to disulfides by oxidation with air or iodine (259).

Sodium hydroxide fusion of the sodium sulfonates of diphenyl ether does not give the desired hydroxy compounds but leads to cleavage, with the formation of sodium phenoxide (259).

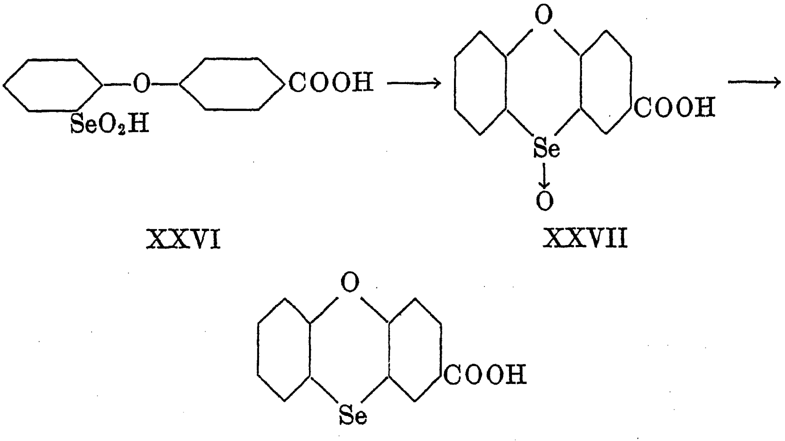
o-Nitrophenoxybenzenesulfonamides (XXV) can rearrange according to the following equation:



XXV

The reaction is inhibited if one of the amide hydrogens is substituted by phenyl (275).

A seleninic acid (XXVI) has been prepared by the diazotization of *p*-(*o*-amino-phenoxy)benzoic acid, replacement of the diazonium group by the selenocyano group, and oxidation with nitric acid (266). Cyclization of this acid with 85 per cent sulfuric acid yields 2-carboxyphenoxaselenin-10-oxide (XXVII), which can be reduced to phenoxaselenin-2-carboxylic acid (XXVIII) by the action of potassium metabisulfite. Attempts to resolve this acid or a dichloro derivative which was also synthesized were unsuccessful (266).



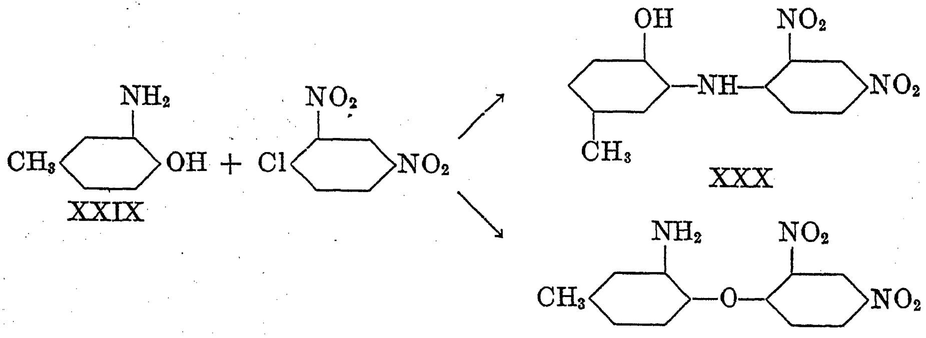
XXVI XXVII

XXVIII

XV. AMINODIPHENYL ETHERS

Amines in this series are prepared mostly by the reduction of the easily accessible nitro compounds, but they can also be obtained directly in good yields through the Ullmann reaction (285). Ullmann and Sponagel report a 57 per cent yield of *m*-phenoxyaniline and a 64 per cent yield of *p*-phenoxyaniline from the reaction of the bromoanilines with potassium phenoxide.

When aminophenols react with activated halogen compounds the reaction can yield either diphenyl ethers or diphenylamines, depending on the reaction conditions. Thus, the aminocresol XXIX reacts with 2,4-dinitrochlorobenzene to give an 85 per cent yield of the diphenylamine XXX when the mixture is heated with alcohol and sodium acetate. When the alcoholic solution of the



XXIX XXX XXXI

sodium salt of XXIX and 2,4-dinitrochlorobenzene is allowed to stand at room temperature, the ether (XXXI) may be isolated in 55 per cent yield (230).

Diamines are prepared by the reduction of dinitro compounds (100) or nitroamines of diphenyl ether (36). The reduction of dinitrodiphenyl ethers may be interrupted at the stage of the intermediate nitroamines if ammonium sulfide (61) or stannous chloride (234) is used as reducing agent.

Two β-(4-aryloxyphenyl)ethylamines have been prepared by the reduction of the corresponding nitrostyrenes (251, 256). Other side-chain amines of interest for the physiological action have been described by Pützer and Schönhöfer (215).

Amines of diphenyl ether are easily acetylated and benzoylated. The resulting amides are useful for identification purposes and permit the study of substitution reactions. The nitration of 2-acetaminodiphenyl ether gives the 5-nitro derivative (181). Chlorination and iodination yield mixtures, but the bromination in acetic acid solution yields the 5-bromo compound (180). In the 4-acetamino isomer the nitro group enters the 3-position. Chlorination leads to a dichloro derivative which contains one chlorine in the 4′-position; bromination and iodination yield the 4′-halogeno derivatives under ordinary conditions (242, 243). When a nitro group is present in the same ring as an acetamino group, additional substituents enter the non-nitrated ring. The substitution reactions of the acetamino compounds in general are governed by two factors,—the strong directive effect of the acetamino group, which exceeds that of any of the other groups considered, and a strong steric factor which tends to inhibit substitution ortho to the acetamino group (180).

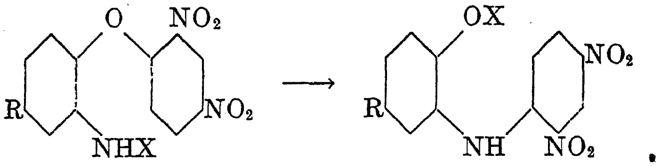
The amino groups in diphenyl ether are easily replaced by halogen by means of the Sandmeyer reaction, which is frequently used to establish the structures of various halogenation products (35). The replacement with the cyano group allows the preparation of acids (258). The amino group may be replaced by hydrogen in good yield when the diazonium salts are reduced with alkaline formaldehyde (33).

The diazonium salts of 2- and 4-aminodiphenyl ethers with chlorines substituted in positions ortho or para to the ether linkage can be salted out of an acid solution. They are stable when dry and mixed with standardizing diluents. These salts are valuable for the production of red and pink color shades with 2-hydroxy-3-naphthoic aryl amides (241).

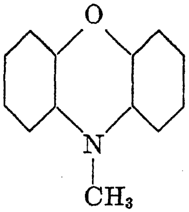
Jones and Cook (138) have described a series of seventy-two dyes derived from *o*- and *p*-aminodiphenyl ethers and their sulfonic acids.

2-Phenoxyanilines can be converted to dibenzofurans by heating their diazonium salts with 50 per cent sulfuric acid (97, 183). The reaction is apparently fairly general (181). It fails to give 4-substituted dibenzofurans unless the amino group is in the same ring as the group which is to be the 4-substituent (87). In the case of *o*-(2,5-dimethoxyphenoxy)aniline the amino group undergoes replacement with hydroxyl rather than cyclization to the dibenzofuran (88).

o-(2,4-Dinitrophenoxy)anilines are unstable to heat in hydroxylic or basic solvents and rearrange to diphenylamines (230):



The rearrangement proceeds readily when X=H and R=NH_2, OCH_3, CH_3, H, Cl, Br, or I, but not when R=COOH or $COOC_6H_3(NO_2)_2$ (232). The reaction takes place more slowly when X=acyl, the velocity being inversely proportional to the acid strength of the acids containing the acyl group (231). In the case of X=CH_3, the rearrangement gave the phenoxazine (XXXII), which was evidently formed by cyclization of the expected rearrangement product. Synthetic *N*-methyl-*o*-hydroxydiphenylamine cyclized to give XXXII under the conditions of the rearrangement (229).



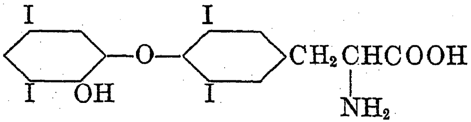
XXXII

o-(2-Hydroxyphenoxy)aniline has been converted to dibenzodioxin by heating the diazonium salt with 50 per cent sulfuric acid and copper sulfate and to phenoxazine, although in small yield, by heating the free amine under pressure (61).

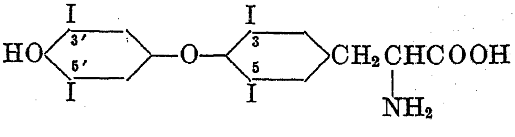
The ether linkage in the three monoamines of diphenyl ether is cleaved by sodium in liquid ammonia. The amino group stabilizes the linkage between oxygen and the aminated ring toward cleavage with this reagent, so that aminophenols are produced in good yield (157, 240, 300).

XVI. AMINO ACIDS

Most of the amino acids containing the diphenyl ether structure have been synthesized with the aim of preparing substances with thyroxine activity. With the exception of the synthesis of "ortho" thyroxine (XXXIII) by Niemann and Mead (200), all such attempts have failed. Thyroxine activity is thus highly specific and limited to thyroxine (XXXIV), the corresponding keto acid (48), and the ortho isomer (XXXIII).



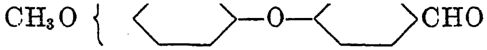
XXXIII



Both of the latter substances, however, possess only a fraction of the activity of thyroxine. The activity is likewise decreased when part or all of the iodine atoms in thyroxine are exchanged for chlorine or bromine (248). The physiological activity disappears when the iodine atoms in the 3- and 5-positions are removed (24), and is greatly decreased in 3,5-diiodothyronine (114).

The elucidation of the structure of thyroxine by Harington (112, 113, 114) was based on the degradation and synthesis of the desiodo compound, thyronine. The location of the iodine atoms was established by alkali fusion of the naturally occurring material and finally by the synthesis of thyroxine (114, 115).

The starting materials for the synthesis of amino acids of this type are the aldehydes (XXXV) or their 3,5-diiodo derivatives.

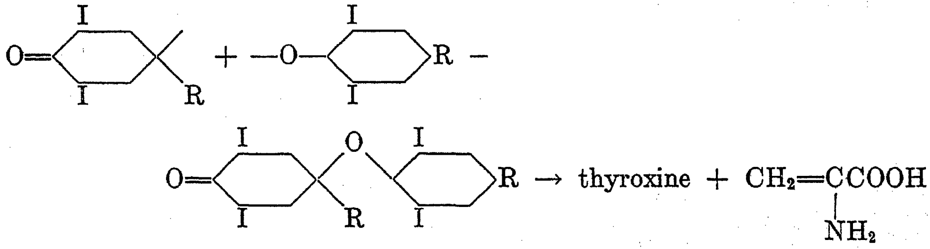


XXXV

The latter are prepared from 3,4,5-triiodonitrobenzene by the Ullmann reaction with appropriate methoxyphenols, and successive replacement of the nitro group with NH_2, CN, and CHO (30, 115, 199, 200, 201, 202). The halogen-free aldehydes are best prepared from the corresponding acids by the McFayden–Stevens method (118, 292).

The amino acids are usually prepared from the aldehydes by the Erlenmeyer synthesis (116). Other procedures involve the use of acetylglycine (30), hydantoin, or glycine anhydride (112).

Thyroxine-containing proteins can be prepared by the iodination of proteins such as casein at 37°C. in the pH range of 7–9 with a limited amount of iodine (177, 197). The intermediate in the reaction is believed to be diiodotyrosine (combined in the protein molecule), since the iodination of casein at lower temperatures causes only substitution of iodine, and because it is possible to produce thyroxine, although in small yield, by the incubation of diiodotyrosine at 37°C. and pH 8.8 (197). These results have been fully substantiated by other workers (23, 117, 137). The mechanism of the reaction proposed by Johnson and Tewkesbury (137) involves the addition of two free radicals derived from diiodotyrosine by dehydrogenation and elimination of one side chain:



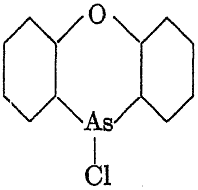
R = $—CH_2CH(NH_2)COOH$

The side-chain fragment was actually isolated in the form of pyruvic acid and ammonia (137). Other possible intermediates in the reaction, such as triiodophenol and 3,5-diiodo-4-hydroxybenzoic acid, have been eliminated (20).

XVII. PHOSPHORUS, ARSENIC, AND TELLURIUM COMPOUNDS

p-Phenoxydichlorophosphine was obtained by Davies and Morris by refluxing diphenyl ether with phosphorus trichloride and aluminum chloride (62). Its structure was established by conversion to tri-*p*-phenoxyphenylphosphine with *p*-phenoxyphenylmagnesium bromide. The same phosphine and methiodide were obtained by synthesis from *p*-phenoxyphenylmagnesium bromide and phosphorus trichloride. An independent proof of structure consisted in brominating the phosphonic acid obtained by hydrolyzing the dichlorophosphine dichloride. Bromine in carbon tetrachloride gave the 4′-bromo acid, which on heating with bromine at 160°C. yielded 4,4′-dibromodiphenyl ether (62). With aliphatic Grignard reagents 4-phenoxyphenyldichlorophosphine yielded dialkylphosphines which gave crystalline addition compounds with carbon disulfide (63).

Arsenic can be introduced into diphenyl ether in the same way as phosphorus, by refluxing with arsenic trichloride and aluminum chloride (169). Substitution in this case occurs in the 2-position, with simultaneous cyclization to give 10-chlorophenoxarsine (XXXVI):

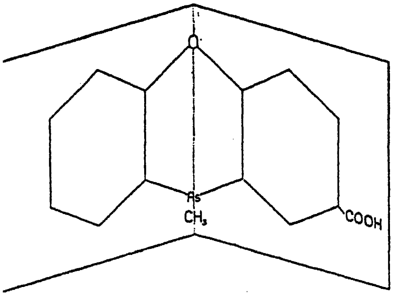


XXXVI

The structure of this chloroarsine has been established by an unambiguous synthesis from 2-aminodiphenyl ether (276) through the Bart reaction. This same reaction is used in the majority of the cases for the preparation of arsonic acids of diphenyl ether (109). It consists in decomposing diazonium salts with sodium arsenite in a buffered solution. The arsonic acids are converted to dichloroarsines by reducing with sulfur dioxide in warm hydrochloric acid in the presence of a little potassium iodide (228). *o*-Aryloxyphenyldichloroarsines are cyclized to chlorophenoxarsines by heating at 200°C. (195, 228). The cyclization rates have been determined for a number of these compounds by measuring the amount of hydrochloric acid produced (195). In some cases *o*-phenoxyphenylarsonic acids can be cyclized by heating with sulfuric acid. When cyclization does not take place, the acids usually sulfonate instead (228).

Chlorophenoxarsines can also be prepared by heating a chloromercuridiphenyl ether with arsenic trichloride and cyclizing the resulting dichloroarsine (195). 8-Chloro-10-phenylphenoxarsine-2-carboxylic acid and 10-methylphenoxarsine-2-carboxylic acid (XXXVII) have been resolved (163, 165). The optical activity of these compounds is due to the fact that the compound is non-planar and arranged in two planes intersecting about the oxygen–arsenic axis.

Arsonic acids of diphenyl ether may be prepared directly by the Ullmann reaction with *o*-chlorophenylarsonic acid and phenoxides, because the arsonic acid group activates the halogen atom (72). 4-Bromo-3-nitrophenylarsonic acid reacts similarly with various phenoxides to give 3-nitro-4-aryloxyarsonic acids (182).

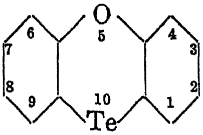


XXXVII

4-Phenoxyphenylarsonic acid is chlorinated and brominated in the 4′-position. The structures of the halogenated products have been established by synthesis from 4′-halo-4-aminodiphenyl ethers (64).

Dimethylarsines have been prepared from 2-phenoxyphenyldichloroarsines by the action of methylmagnesium iodide, and from 4-phenoxyphenylmagnesium bromide and iododimethylarsine. They can be characterized as methiodides (64).

Tellurium is introduced into diphenyl ether by treating the ether with tellurium tetrachloride in chloroform solution. At low temperatures the product is *p*-phenoxyphenyltelluriumtrichloride. When this is heated to 200°C., or when the original reaction mixture is heated to 200°C., 10,10-dichlorophenoxatellurin is produced (68). Reduction of the dichloride with potassium metabisulfite at 0°C. furnishes phenoxatellurin (XXXVIII) in excellent yield.



XXXVIII

Phenoxatellurin

On nitration with concentrated nitric acid XXXVIII yields a mixture of 4- and 8-nitro compounds. Fuming nitric acid produces the 4,8- and 2,8-dinitrotellurins. The nitro compounds readily lose tellurium when boiled with aqueous potassium hydroxide (69).

o-Tolyloxyphenyltelluriumtrichloride has been prepared from the chloromercuri compound by the action of tellurium tetrachloride (47). Attempts to resolve phenoxatellurin-2-carboxylic acid have been unsuccessful.

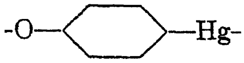
XVIII. MERCURY, LITHIUM, AND SODIUM COMPOUNDS

Mercuration of diphenyl ether with mercuric acetate in acetic acid at 100°C. was found to give approximately equal amounts of 4-acetoxymercuridiphenyl ether and 4,4′-diacetoxymercuridiphenyl ether (247). The ratio of these products was about the same when the reaction was carried out at room temperature. 4-Bromo- and 4-iodo-diphenyl ethers were mercurated in the 4′-position by this method, while 4-hydroxydiphenyl ether gave a mixture of polymercury compounds. Individual mercury compounds could not be obtained from 4-methoxydiphenyl ether, but 4-benzoyloxydiphenyl ether gave the 4′-acetoxymercuri derivative (247).

The mercuri group in the pure acetoxymercuri compounds is replaced with iodine upon treatment with iodine monochloride in acetic acid solution. 4-Acetoxymercuridiphenyl ether reacts in the normal manner with warm mineral acids to give diphenyl ether, with sodium chloride solution to give 4-chloromercuridiphenyl ether, and with bromine to give 4-bromodiphenyl ether. In 4,4′-diacetoxymercuridiphenyl ether both groups are replaced by these same reagents with hydrogen, chloromercuri groups, or bromine, respectively (247). Chloromercuri compounds are also obtained in good yield by the decomposition of diazomercuric chlorides of diphenyl ether with copper powder (47).

Reactive alkyl halides and aroyl halides have been found to react with 4-chloromercuridiphenyl ether at 150°C. to give mercuric halides and alkylated or acylated diphenyl ethers. Benzylation yields 4-benzyl- and 4,4′-dibenzyldiphenyl ethers in this manner. In order to explain the formation of the dibenzyl compound, Schroeder and Brewster assume that the mercuric chloride which is liberated in the reaction between benzyl chloride and the chloromercuri compound mercurates the benzyldiphenyl ether in the 4′-position. Subsequent benzylation would lead to the 4,4′-dibenzyl compound. In support of this theory the authors were able to benzylate diphenyl ether with benzyl chloride and mercuric chloride. The two benzylation products were formed in approximately the same ratio as in the previous reaction (247). The rates of benzylation for diphenyl ether, *p*-bromodiphenyl ether, and *p*-nitrodiphenyl ether with mercuric chloride were in the same order as the rates of bromination for the same compounds. The reaction was of the first order, indicating that the rate-determining step was the speed of dissociation of a coördination compound which may be formulated as in the Friedel–Crafts reaction (34). Benzoyl chloride and *tert*-amyl chloride react with diphenyl ether and mercuric chloride to give the corresponding 4-substituted derivatives (247). Anhydrous cadmium chloride and zinc chloride are said to be even more efficient than mercuric chloride in reactions of this type with diphenyl ether (247).

4-Mercuribis(diphenyl ether) (XXXIX) is formed from 4-bromodiphenyl



XXXIX

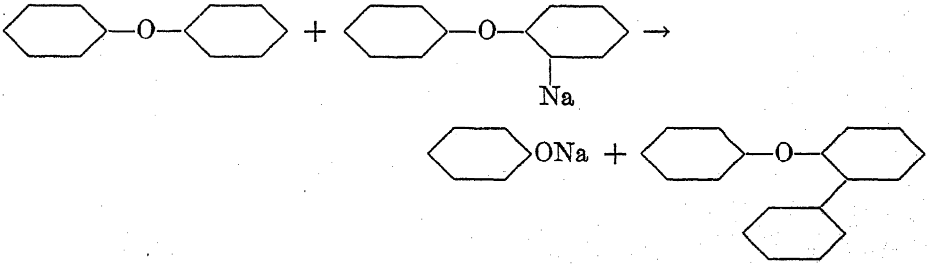
ether by heating with sodium amalgam in toluene containing some ethyl acetate at 120°C. (247) or by the action of mercuric chloride on 4-phenoxyphenylmagnesium bromide (62). The mercury compound (XXXIX) yields 4-acetoxydiphenyl ether when boiled with acetic acid, and 4-iododiphenyl ether on treatment with iodine monochloride (247).

Metalation with lithium occurs in fairly good yields when diphenyl ether is stirred with *n*-butyllithium in ether solution (85). The position of the metal has been established by isolation of 2-phenoxybenzoic acid as the carbonation product. The orientation of the metal to the 2-position in this case is quite unusual, since all other substitution reactions and also the mercuration of diphenyl ether involve the 4-position.

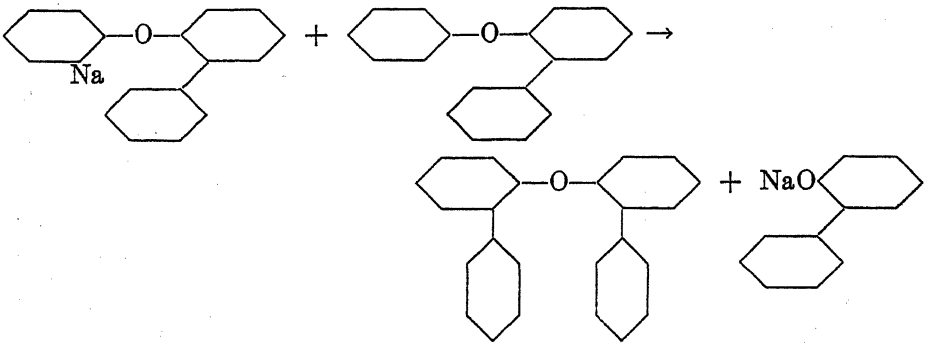
p-Bromodiphenyl ether undergoes metalation with *n*-butyllithium or metallic lithium in the 2-position, as evidenced by the formation of 2-phenoxy-5-bromobenzoic acid on carbonation. The reaction is interpreted as an autometalation involving the intermediate *p*-phenoxyphenyllithium, which is formed by halogen–lithium interchange (86). Further investigations have shown that under mild conditions iodo- and bromo-diphenyl ethers undergo mainly halogen–lithium interconversion, regardless of the position of the halogen. Iododiphenyl ethers give the reaction more readily than bromodiphenyl ethers, while chlorodiphenyl ethers are essentially unaffected. Under more drastic conditions, i.e., during during long periods of reaction or on refluxing in ether solution, the *p*-halogenodiphenyl ethers are metalated by *n*-butyllithium and phenyllithium (158).

4-Methoxydiphenyl ether is metalated at least in part in the 3-position, since 2-methoxy-5-phenoxybenzoic acid has been isolated from the crude mixture of the carbonation acids. The same acid has been obtained from the carbonation of the reaction mixture from 3-bromo-4-methoxydiphenyl ether and *n*-butyllithium (158).

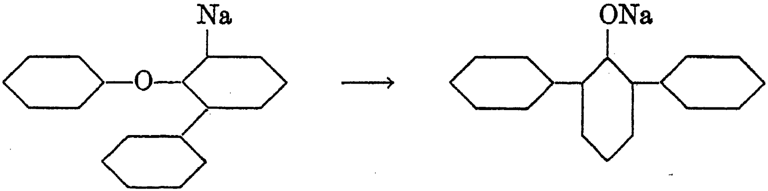
Treatment of diphenyl ether with ethynylsodium in liquid ammonia (85) or heating with ethylmagnesium bromide gives some *o*-phenylphenol (253). This same product has also been isolated from the reaction of diphenyl ether with phenylsodium (179), and its formation may probably be regarded as evidence for intermediate metalation products in these reactions. The existence of a metallic intermediate has been pretty well established in the last case, because carbonation of the reaction mixture yields an acid which has been identified as *o*-phenoxybenzoic acid. The end-products of the reaction in the order of decreasing yields are *o*-phenylphenol, phenol, *o*-phenoxydiphenyl, 2,2′-diphenyldiphenyl ether, and 2,6-diphenylphenol. Lüttringhaus and Säáf have shown that the phenol is derived exclusively from the diphenyl ether by cleavage with *o*-phenoxyphenylsodium:



o-Phenylphenol may result from the cleavage of *o*-phenoxydiphenyl with either *o*-phenoxyphenylsodium or phenylsodium. The latter reaction could be demonstrated experimentally. The mechanism, which assumes an intermediate sodium derivative of *o*-phenoxydiphenyl, also accounts for the formation of 2,2′-diphenyldiphenyl ether, which occurs in equivalent amounts. It is written as follows:

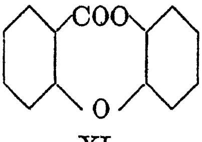


The formation of 2,6-diphenylphenol is postulated as a rearrangement of a third metallic intermediate (179):



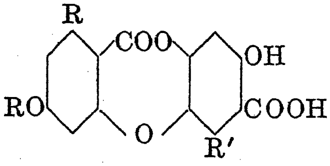
XIX. DEPSIDONES

Depsidones are substituted phenyl benzoates (depsides) in which the two nuclei are linked by an oxygen bridge so that a seven-membered ring is formed (7, 8). The basic ring system is therefore the lactone of *o*-(*o*-hydroxyphenoxy)benzoic acid (XL):

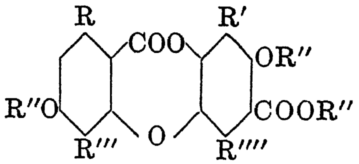


XL

The depsidones occur naturally along with depsides in various lichens. They may be grouped into two classes (XLI and XLII), depending on whether they are derived from homologs of orcinol or of β-orcinol (7).



XLI

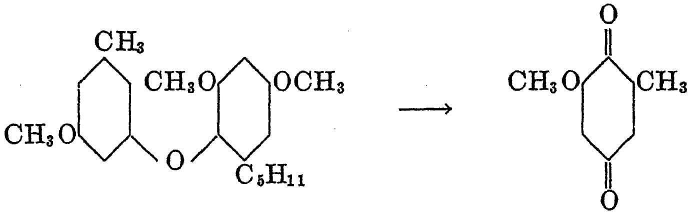


XLII

Depsidones of the first type (XLI) are distinguished by the length of the carbon chains R and R′ in the phenolic nuclei, which may contain one, three, five, or seven carbon atoms. These side chains are either paraffin chains or else contain a keto group in the alpha or beta position.

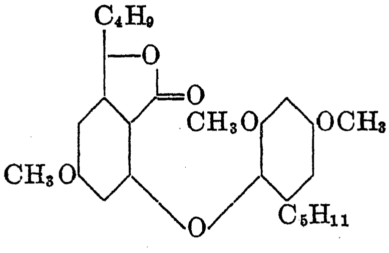
Mild hydrolysis usually opens the lactone ring in depsidones, while boiling with strong alkali or barium hydroxide causes the elimination of carbon dioxide. The ether linkage is broken by alkali fusion. The resulting fragments can be identified for proof of structure. In the case of lobaric acid, where alkali fusion fails to give fragments of both rings, thermal decomposition causes cleavage of the ether linkage with formation of two identifiable products (16).

The diphenyl ether skeleton of the compounds is usually obtained by methylation of all free hydroxyl groups and removal of the carboxyl groups by heating with strong alkali. The structure of these diphenyl ether skeletons can be ascertained from the products obtained on oxidation of the ether and of its bromination product. Protophysodon trimethyl ether (XLIII), for example, gives 6-methoxy-2-methyl-1,4-benzoquinone (XLIV) on oxidation with chromic acid, while its tribromo derivative yields 6-methoxy-2-amyl-3-bromo-1,4-benzoquinone (11).

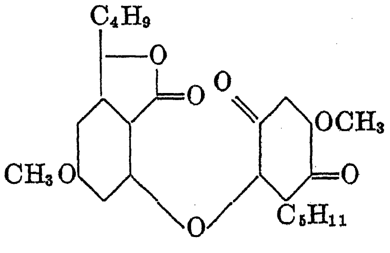


XLIII XLIV

In the oxidation of the dimethyl ether of lobariolide (XLV), a degradation product of lobaric acid, chromic acid oxidation leaves the ether linkage intact, so that the complex aryloxyquinone (XLVI) may be isolated (16). 6-Methoxy-2-amyl-1,4-benzoquinone is also formed on oxidation.



XLV



XLVI

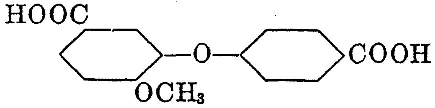
The final proof for the structure of the diphenyl ether skeletons, which are polyalkylpolymethoxydiphenyl ethers, is by synthesis, which has been realized in a number of cases (9, 12, 13, 17).

The phenolic nuclei in the depsidones of the second type (XLII) are characterized by methyl side chains (R, R′, R‴, R⁗) in various stages of oxidation, that is, methyl, methylol, formyl, and carboxyl groups (7). All depsidones of this group known at the present time contain one aldehyde group which gives rise to various characteristic color tests.

For the determination of structures in this class of compounds the various methylol, formyl, and methylol ether groups are reduced to methyl groups (10, 14). The hydrogenated products are then investigated like the depsidones of the first type (9). Additional information can be obtained by submitting depsidones of the β-orcinol type to alkali fusion or pyrolysis.

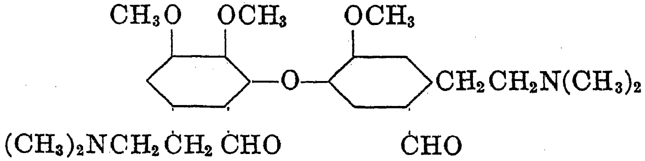
XX. ALKALOIDS

Diphenyl ether linkages occur in the isoquinoline alkaloids of the Berberidaceae, Menispermaceae, papeira bark, and curare (122). Examples of the alkaloids which occur in the first two groups are oxyacanthine and dauricine. Both are somewhat related and yield the same acid (XLVII) when the ammonium base obtained from the exhaustive methylation of the alkaloid is oxidized with potassium permanganate. The structure of the acid (XLVII) has been established by synthesis (155, 239, 254).



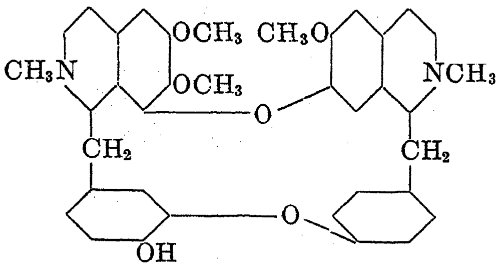
XLVII

A second fragment (XLVIII) of oxyacanthine was obtained along with the dialdehyde corresponding to XLVII by ozonizing the methine base formed in the first stage of the Hofmann degradation (40).

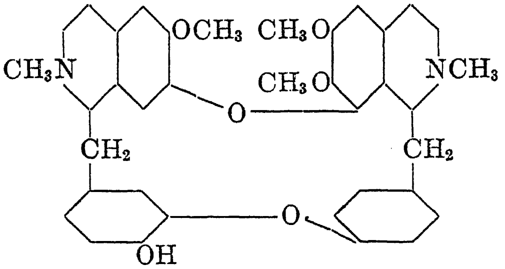


XLVIII

Treatment of the diamine (XLVIII) with alkali gave a nitrogen-free divinyldialdehyde which was reduced to 2,3,2′-trimethoxy-5′,6-dimethyl-4′,5-diethyldiphenyl ether. The structure of the latter was confirmed by synthesis. On the basis of these and other data oxyacanthine was assigned the configuration XLIX or L.

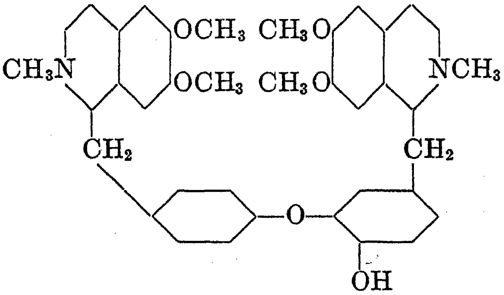


XLIX

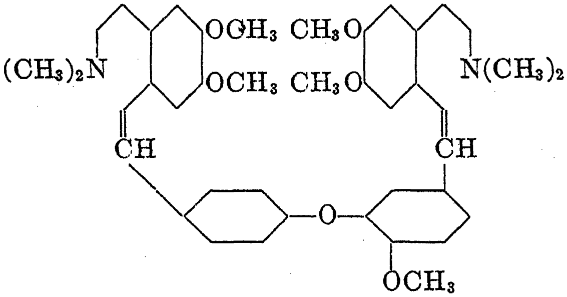


L

The structure of dauricine (LI) is based largely on the synthesis of the methine (LII) which is produced in the first stage of the Hofmann degradation of this alkaloid.

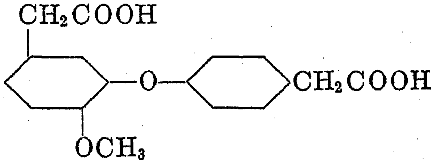


LI



LII

The starting material for this synthesis, 4′,5-diformyl-2-methoxydiphenyl ether, was obtained by the Rosenmund reduction of the corresponding diacid chloride. It was converted to the di(carboxymethyl)diphenyl ether (LIII) by way of the azlactone and diketo acid. The diacid (LIII) gave a diamide with homoveratryl-

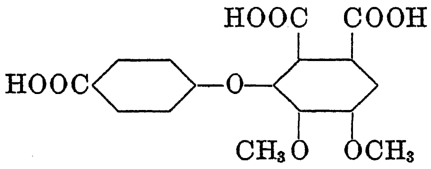


LIII

amine which was cyclized to the bisisoquinoline derivative by treatment with phosphorus pentachloride. Reduction followed by methylation yielded the methine (LII) (155).

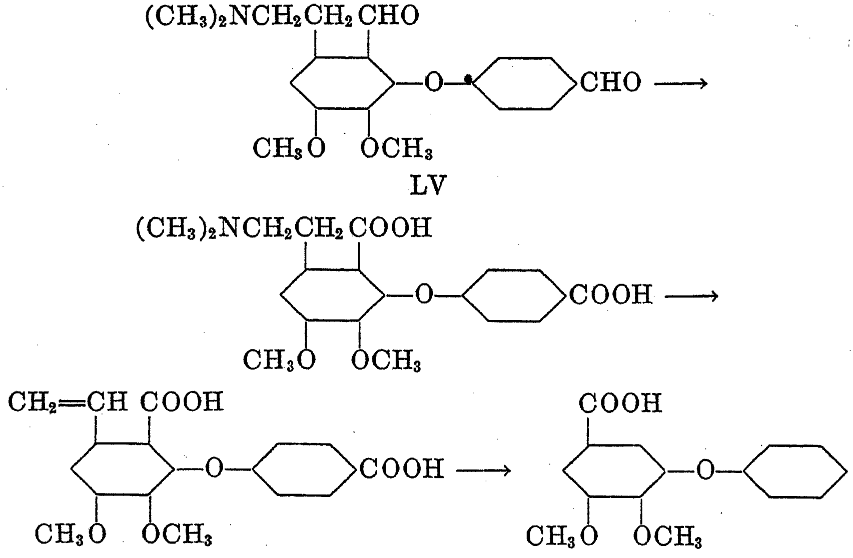
Alkali fusion of oxyacanthine yields *p*-hydroxybenzoic acid (254). The same reaction with tetrandrine and trilobine, two of the Menispermaceae alkaloids, furnishes protocatechuic acid (122).

The two papeira bark alkaloids isochondrodendrine and bebeerine give the same acid (LIV) on oxidation of the non-nitrogenous compounds obtained from the product of the exhaustive methylation of the alkaloids with base. The structure of LIV was established by synthesis (74, 76).



LIV

The structure of isochondrodendrine was based on the above degradation and the ozonization product (LV) of the methine base obtained by exhaustive methylation of the alkaloid. The dialdehyde (LV) was oxidized, deaminated with alkali, decarboxylated, and oxidized to the acid (LVII) which was also prepared from methyl bromoveratrate by the Ullmann reaction (73).

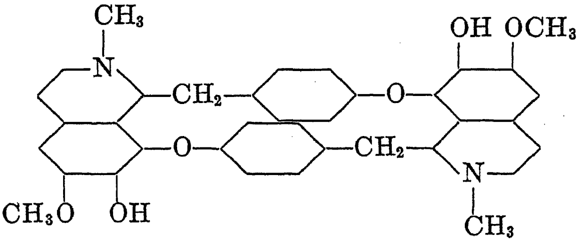


LV

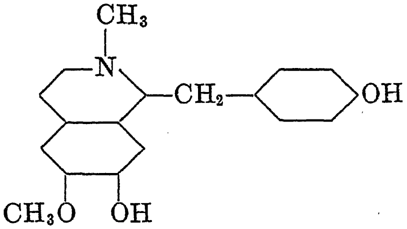
LVI

LVII

In view of the excellent yield of the vinyl acid (LVI) in this degradation, isochondrodendrine was given the structure LVIII, which is built up from two identical fragments, and might be considered as a dehydrogenation product from two molecules of coclaurine (LIX), an alkaloid of the Menispermaceae.

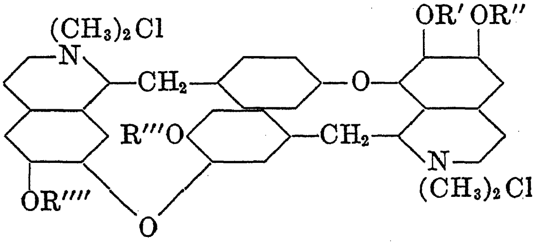


LVIII



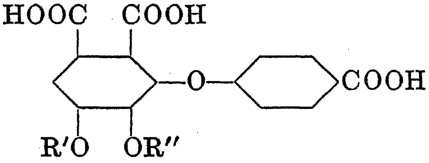
LIX

Bebeerine methochloride and the curare alkaloid tubocurarine chloride are diastereoisomers which have been assigned the structure LX largely on the basis of the oxidation acids (LXI and LXII, where the R groups are methyl)

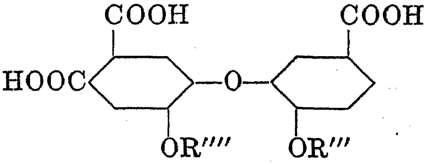


LX

obtained from the deaminated base which results from the exhaustive methylation of the alkaloids.



LXI



LXII

Two of the R groups in LX are methyl and two are hydrogen. The positions of the free hydroxyl groups were determined by King (142), who marked them by ethylation with ethyl iodide. The ethoxy acids (LXI: R′ = CH_3, R″ = CH_2CH_3; LXII: R‴ = CH_2CH_3, R⁗ = CH_3) were prepared by synthesis from the potassium salt of methyl *p*-hydroxybenzoate and methyl 3-bromo-5-methoxy-4-ethoxyphthalate, and the potassium salt of methyl 3-hydroxy-4-ethoxy-benzoate and methyl 5-iodo-4-methoxyphthalate. Since they were found to be identical with the degradation acids, R'' and R''' in the alkaloids LX must be hydrogens, and R' and R'''' must be methyl groups.

The methods used for the structural elucidation of other alkaloids containing diaryl ether linkages are very similar to those discussed in the preceding examples.

The author has made use of the historical part of a thesis submitted by E. F. Orwoll to the Faculty of the Graduate School of the University of Missouri in partial fulfilment of the requirements for the degree of Doctor of Philosophy in August, 1945.

XXI. REFERENCES

(1) Adams, R., and Montgomery, E.: J. Am. Chem. Soc. **46**, 1518 (1924).
(2) Adkins, H.: *Reactions of Hydrogen*, p. 59. The University of Wisconsin Press, Madison (1937).
(3) Andersag, H.: *Medizin und Chemie*, Vol. 2, p. 359. I. G. Farbenindustrie, Elberfeld (1934).
(4) Amatatsu, R., and Araki, S.: J. Chem. Soc. Japan **52**, 484 (1931).
(5) Anschütz, R., Stoltenhoff, W., and Voeller, F.: Ber. **58B**, 1736 (1925).
(6) Arbenz, C.: Ann. **257**, 76 (1890).
(7) Asahina, Y.: Acta Phytochim. (Japan) **8**, 33 (1934).
(8) Asahina, Y.: Fortschr. Chem. org. Naturstoffe **2**, 46 (1939).
(9) Asahina, Y., and Asano, J.: Ber. **66**, 1215 (1933).
(10) Asahina, Y., and Hayashi, H.: Ber. **70**, 810 (1937).
(11) Asahina, Y., and Nogami, H. Ber. **68**, 77 (1935).
(12) Asahina, Y., and Nogami, H. Ber. **68**, 1500 (1935).
(13) Asahina, Y., and Shibata, S.: Ber. **72**, 1399 (1939).
(14) Asahina, Y., and Tanase, Y.: Ber. **67**, 766 (1934).
(15) Asahina, Y., and Tanase, Y.: Proc. Imp. Acad. (Tokyo) **16**, 297 (1940).
(16) Asahina, Y., and Yasue, M.: Ber. **69**, 644 (1936).
(17) Asahina, Y., and Yasue, M.: Ber. **70**, 206 (1937).
(18) Aylsworth, J. W.: U. S. patent 1,213,142 (January 23, 1917).
(19) Baeyer, A. v.: Ann. **372**, 99 (1910).
(20) Barkdoll, A. E., and Ross, W. F.: J. Am. Chem. Soc. **66**, 898 (1944).
(21) Barnett, E. B., and Goodway, N. F.: Ber. **63**, 3048 (1930).
(22) Belov, V. N., and Shepelenkova, E. I.: J. Gen. Chem. (U.S.S.R.) **11**, 757 (1941).
(23) Block, P., Jr.: J. Biol. Chem. **135**, 51 (1940).
(24) Block, P., Jr., and Powell, G.: J. Am. Chem. Soc. **64**, 1070 (1942).
(25) Böhmer, C.: J. prakt. Chem. [2] **24**, 473 (1881).
(26) Bonneaud, M. A.: Bull. soc. chim. [4] **7**, 779 (1910).
(27) Borsche, W.: Ber. **56**, 1488 (1923).
(28) Bost, R. W., and Nicholson, F.: J. Am. Chem. Soc. **57**, 2368 (1935).
(29) Bouveault, M. L.: Bull. soc. chim. [3] **17**, 949 (1897).
(30) Bovarnick, M., Bloch, K., and Foster, G. L.: J. Am. Chem. Soc. **67**, 562 (1945).
(31) Branch, G. E. K., and Calvin, M.: *The Theory of Organic Chemistry*, p. 137. Prentice-Hall, Inc., New York (1941).
(32) Brewster, R. Q., and Choguill, H. S.: J. Am. Chem. Soc. **61**, 2702 (1939).
(32a) Brewster, R. Q., and Groening, T.: *Organic Syntheses*, Vol. 14, p. 66. John Wiley and Sons, Inc., New York (1934).

(33) Brewster, R. Q., and Poje, J. A.: J. Am. Chem. Soc. **61**, 2419 (1939).
(34) Brewster, R. Q., and Slocombe, R.: J. Am. Chem. Soc. **67**, 562 (1945).
(35) Brewster, R. Q., and Stevenson, G.: J. Am. Chem. Soc. **62**, 3144 (1940).
(36) Brewster, R. Q., and Strain, F.: J. Am. Chem. Soc. **56**, 117 (1934).
(37) Briner, E., Bron-Stalet, J., and Paillard, H.: Helv. Chim. Acta **15**, 619 (1932).
(38) Britton, E. C.: U. S. patent 1,959,283 (May 15, 1934).
(39) Britton, E. C., Stoesser, W. C., and Goergen, G. G.: U. S. patent 2,022,634 (November 26, 1935).
(40) Bruchhausen, F. v., Oberempt, H., and Feldhaus, A.: Ann. **507**, 145 (1933).
(41) Buch, K.: Ber. **17**, 2634 (1884).
(42) Buchan, S., and Scarborough, H. A.: J. Chem. Soc. **1934**, 705.
(43) Cahn, S.: J. Chem. Soc. **1931**, 1121.
(44) Calcott, W. S., Douglass, W. A., and Walker, H. W.: U. S. patent 1,913,368 (1933).
(45) Calcott, W. S., Tinker, J. M., and Weinmayr, V.: J. Am. Chem. Soc. **61**, 1014 (1939).
(46) Campbell, N. R., and Chattaway, F. W.: Proc. Roy. Soc. (London) **B130**, 435 (1942).
(47) Campbell, I. G. M., and Turner, E. E.: J. Chem. Soc. **1938**, 37.
(48) Canzanelli, A., Guild, R., and Harington, C. R.: Biochem. J. **29**, 1617 (1935).
(49) Clarkson, R. G., and Gomberg, M.: J. Am. Chem. Soc. **52**, 2881 (1930).
(50) Coleman, G. H., and Dreisbach, R. D.: U. S. patent 2,170,989 (August 29, 1939).
(51) Coleman, G. H., and Hadler, B. C.: U. S. patent 2,079,279 (May 4, 1937).
(52) Coleman, G. H., and Perkins, R. P.: U. S. patent 2,170,809 (August 29, 1939).
(53) Cook, A. N.: J. Am. Chem. Soc. **25**, 61 (1903).
(54) Cook, A. N.: J. Am. Chem. Soc. **26**, 302 (1904).
(55) Cook, A. N.: Am. Chem. J. **36**, 543 (1906).
(56) Cook, A. N.: J. Am. Chem. Soc. **32**, 1285 (1910).
(57) Cook, A. N., and Eberly, C. F.: J. Am. Chem. Soc. **24**, 1200 (1902).
(58) Cook, A. N., and Frary, G. G.: Am. Chem. J. **28**, 486 (1902).
(59) Cook, A. N., and Sherwood, F. F.: J. Am. Chem. Soc. **37**, 1835 (1915).
(60) Cox, E. H.: J. Am. Chem. Soc. **52**, 352 (1930).
(61) Cullinane, N. M., Davey, H. G., and Padfield, H. J. H.: J. Chem. Soc. **1934**, 716.
(62) Davies, W. C., and Morris, C. J. O. R.: J. Chem. Soc. **1932**, 2880.
(63) Davies, W. C., and Morris, C. J. O. R.: Bull. soc. chim. [4] **53**, 980 (1933).
(64) Davies, W. C., and Othen, C. W.: J. Chem. Soc. **1936**, 1236.
(65) Dilthey, W., Bach, E., Grütering, H., and Hausdörfer, E.: J. prakt. Chem. **117**, 337 (1927).
(66) Dilthey, W., and Harenberg, F.: J. prakt. Chem. **136**, 67 (1933).
(67) Dilthey, W., Neuhaus, L., Reis, E., and Schommer, W.: J. prakt. Chem. **124**, 81 (1929).
(68) Drew, H. D. K.: J. Chem. Soc. **1926**, 223.
(69) Drew, H. D. K., and Thomason, R. W.: J. Chem. Soc. **1927**, 116.
(70) Eckert, A., and Seidel, F.: J. prakt. Chem. [2] **102**, 344 (1921).
(71) Etard, M. A.: Bull. soc. chim. [2] **28**, 276 (1877).
(72) Etzelmiller, R. E., and Hamilton, C. S.: J. Am. Chem. Soc. **53**, 3085 (1931).
(73) Faltis, F., and Dieterich, H.: Ber. **67B**, 231 (1934).
(74) Faltis, F., and Frauendorfer, H.: Ber. **63**, 806 (1930).
(75) Faltis, F., Holzinger, L., Ita, P., and Schwarz, R.: Ber. **74B**, 79 (1941).
(76) Faltis, F., and Troller, A.: Ber. **61B**, 345 (1928).
(77) Fichter, F., and Brunner, E.: Bull. soc. chim. [4] **19**, 280 (1916).
(78) Fosse, R.: Bull. soc chim [3] **31**, 253 (1904).
(79) Fosse, R., and Robyn, A.: Bull. soc. chim. [3] **31**, 267 (1904).
(80) Fox, D. L., and Turner, E. E.: J. Chem. Soc. **1930**, 1115.
(81) Fox, D. L., and Turner, E. E.: J. Chem. Soc. **1930**, 1853.

(82) Frey, M.: Ber. **45**, 1359 (1912).
(83) Fuzikawa, F.: Ber. **68**, 73 (1935).
(84) Gattermann, L.: Ann. **357**, 363 (1907).
(85) Gilman, H., and Bebb, R. L.: J. Am. Chem. Soc. **61**, 109 (1939).
(86) Gilman, H., Langham, W., and Jacoby, A. L.: J. Am. Chem. Soc. **61**, 106 (1939).
(87) Gilman, H., Van Ess, M. W., and Hayes, D. M.: J. Am. Chem. Soc. **61**, 643 (1939).
(88) Gilman, H., and Van Ess, P. R.: J. Am. Chem. Soc. **61**, 1365 (1939).
(89) Gladstone, J. H., and Tribe, A.: J. Chem. Soc. **41**, 5 (1882).
(90) Gladstone, J. H., and Tribe, A.: J. Chem. Soc. **49**, 27 (1886).
(90a) Goldschmidt, S.: *Hand-und Jahrbuch der chemischen Physik*, Vol. 4, p. 161. Akademische Verlagsgesellschaft, Leipzig (1933).
(91) Goldschmidt, A., Schultz, E., and Bernard, H.: Ann. **478**, 1 (1931).
(92) Gomberg, M., and Cone, L. H.: Ann. **370**, 183 (1909).
(93) Gottesman, E.: Ber. **66**, 1168 (1933).
(94) Graebe, C.: Ber. **13**, 1851 (1880).
(95) Graebe, C.: Ann. **209**, 150 (1881).
(96) Graebe, C.: Ber. **21**, 501 (1888).
(97) Graebe, C., and Ullmann, F.: Ber. **29**, 1876 (1896).
(98) Griess, P.: Ber. **21**, 980 (1888).
(99) Groves, L. G., Turner, E. E., and Sharp, G. I.: J. Chem. Soc. **1929**, 512.
(100) Haeusserman, C., and Bauer, E.: Ber. **29**, 2085 (1896).
(101) Haeusserman, C., and Bauer, E.: Ber. **30**, 738 (1897).
(102) Haeusserman, C., and Müller, A.: Ber. **34**, 1070 (1901).
(103) Haeusserman, C., and Schmidt, O.: Ber. **34**, 3770 (1901).
(104) Haeusserman, C., and Teichmann, H.: Ber. **29**, 1446 (1896).
(105) Hale, W. J., and Britton, E. C.: U. S. patent 1,737,841 (December 3, 1929).
(105a) Hale, W. J., and Britton, E. C.: U. S. patent 1,737,842 (December 13, 1929).
(106) Hale, W. J., and Britton, E. C.: U. S. patent 1,806,798 (May 26, 1931).
(107) Hale, W. J., and Britton, E. C.: U. S. patent 1,882,824 (October 18, 1933).
(108) Hale, W. J., and Britton, E. C.: U. S. patent 1,925,321 (September 5, 1933).
(109) Hamilton, C. S., and Morgan, J. F.: In *Organic Reactions*, Vol. 2, p. 417. John Wiley and Sons, Inc., New York (1944).
(110) Hammett, L. P.: *Physical Organic Chemistry*, p. 33. McGraw-Hill Book Co., Inc., New York and London (1940).
(111) Hampson, G. C., Farmer, R. H., and Sutton, L. E.: Proc. Roy. Soc. (London) **A143**, 150 (1933).
(112) Harington, C. R.: Biochem. J. **20**, 300 (1926).
(113) Harington, C. R.: *The Thyroid Gland*, pp. 93–111. Oxford University Press, London (1933).
(114) Harington, C. R.: Fortschr. Chem. org. Naturstoffe **2**, 103 (1939).
(115) Harington, C. R., and Barger, G.: Biochem. J. **21**, 169 (1927).
(116) Harington, C. R., and McCartney, W.: Biochem. J. **21**, 852 (1927).
(117) Harington, C. R., and Rivers, R. P.: Nature **144**, 205 (1939).
(118) Harington, C. R., and Rivers, R. P.: J. Chem. Soc. **1940**, 1101.
(119) Heindel, L.: Trans. Am. Inst. Chem. Engrs. **32**, 73 (1936).
(120) Henley, R. V.: J. Chem. Soc. **1930**, 1222.
(121) Henley, R. V., and Turner, E. E.: J. Chem. Soc. **1930**, 928.
(122) Henry, T. A.: *The Plant Alkaloids*, pp. 352–83. P. Blakiston's Son and Co., Philadelphia (1939).
(123) Hillyer, H. W.: Am. Chem. J. **26**, 365 (1901).
(124) Hirsch, R.: Ber. **23**, 3705 (1890).
(125) Hirsch, R.: German patent 58,001 (June 10, 1890).
(126) Hoffmeister, W.: Ber. **3**, 747 (1870).
(127) Hoffmeister, W.: J. prakt. Chem. [2] **1**, 143 (1870).

(128) Hoffmeister, W.: Ann. **159**, 191 (1871).
(129) Hönigschmid, O.: Monatsh. **23**, 824 (1902).
(130) Houben, J., and Fischer, W.: Ber. **64**, 2645 (1931).
(131) Hückel, W.: *Theoretische Grundlagen der organischen Chemie*, 2nd edition, p. 55. Akademische Verlagsgesellschaft, Leipzig (1935).
(132) Huntress, E. H., and Carten, F. H.: J. Am. Chem. Soc. **62**, 603 (1940).
(133) Ipatieff, W., and Orlow, N.: Ber. **60B**, 1963 (1927).
(134) Ipatieff, W., and Philipov, O.: Ber. **41**, 1001 (1908).
(135) Japp, F. R., and Findlay, A. J.: J. Chem. Soc. **71**, 1119 (1879).
(136) Jeiteles, B.: Monatsh. **17**, 65 (1896).
(137) Johnson, T. B., and Tewkesbury, L. B.: Proc. Natl. Acad. Sci. U. S. **28**, 73 (1942).
(138) Jones, H. I., and Cook, A. N.: J. Am. Chem. Soc. **38**, 1535 (1916).
(139) Keimatsu, I., and Yamaguchi, E.: J. Pharm. Soc. Japan **56**, 680 (1936).
(140) Kent, B. A., and Smiles, S.: J. Chem. Soc. **1934**, 422.
(141) King, F. E.: J. Chem. Soc. **1934**, 1064.
(142) King, H.: J. Chem. Soc. **1939**, 1157.
(143) King, H.: J. Chem. Soc. **1939**, 1165.
(144) King, H., and Wright, E. V.: J. Chem. Soc. **1939**, 1168.
(145) Kipper, H.: Ber. **38**, 2490 (1905).
(146) Klarman, E., Gatyas, L. W., and Shternov, V. A.: J. Am. Chem. Soc. **53**, 3404 (1931).
(147) Klarman, E., Gatyas, L. W., and Shternov, V. A.: J. Am. Chem. Soc. **54**, 298 (1932).
(148) Klepl, A.: J. prakt. Chem. [2] **28**, 199 (1883).
(149) Knapp, W. A.: U. S. patent 2,251,743 (August 5, 1941).
(150) Kohler, E. P.: Am. Chem. J. **27**, 241 (1902).
(151) Kohler, E. P., Heritage, G. L., and Burnley, M. C.: Am. Chem. J. **44**, 60 (1910).
(152) Komatsu, S., and Masumoto, M.: Bull. Chem. Soc. Japan **5**, 241 (1930).
(153) Komatsu, S., Sugino, K., and Hagiwara, M.: Proc. Imp. Acad. (Tokyo) **6**, 194 (1930).
(154) Kondo, H., and Keimatsu, I.: Ber. **71**, 2553 (1938).
(155) Kondo, H., Narita, Z., and Uyeo, S.: Ber. **68**, 519 (1935).
(156) Krafft, F., and Steiner, O.: Ber. **34**, 561 (1901).
(157) Kranzfelder, A. L., Verbanc, J. J., and Sowa, F. J.: J. Am. Chem. Soc. **59**, 1488 (1937).
(157a) Krishna, S.: J. Chem. Soc. **123**, 2782 (1923).
(158) Langham, W., Brewster, R. Q., and Gilman, H.: J. Am. Chem. Soc. **63**, 545 (1941).
(159) Laube, E.: Ber. **39**, 2245 (1906).
(160) Lea, T. R., and Robinson, R.: J. Chem. Soc. **1926**, 411.
(161) LeFevre, R. J. W., Saunders, S. L. M., and Turner, E. E.: J. Chem. Soc. **1927**, 1168.
(162) Lespagnol, A., Bertrand, J., and Dupas, J.: Bull. soc. chim. [5] **6**, 1625 (1939).
(163) Lesslie, M. S.: J. Chem. Soc. **1938**, 1001.
(164) Lesslie, M. S., and Turner, E. E.: J. Chem. Soc. **1932**, 281.
(165) Lesslie, M. S., and Turner, E. E.: J. Chem. Soc. **1934**, 1170.
(166) Levi, A. A., and Smiles, S.: J. Chem. Soc. **1932**, 1488.
(167) Levy, A. A., Rains, H. C., and Smiles, S.: J. Chem. Soc. **1931**, 3264.
(168) Lewis, W. L., and Cheetham, H. C.: J. Am. Chem. Soc. **43**, 2117 (1921).
(169) Lewis, W. L., Lowry, C. D., and Bergeim, F. H.: J. Am. Chem. Soc. **43**, 891 (1921).
(170) Lions, F., and Willison, A. M.: J. Proc. Roy. Soc. N. S. Wales **71**, 435 (1938).
(171) Lions, F., and Willison, A. M.: J. Proc. Roy. Soc. N. S. Wales **72**, 257 (1939).
(172) List, K., and Limpricht, H.: Ann. **90**, 209 (1854).
(173) Lobry de Bruyn, C. A., and VanGeuns, J. W.: Rec. trav. chim. **23**, 26 (1904).
(174) Lock, G. M.: Monatsh. **55**, 167 (1930).

(175) Lock, G., and Kempter, F. H.: Monatsh. **67**, 24 (1935).
(176) Loewenich, J., Becker, W., and Schröder, T.: J. prakt. Chem. **127**, 248 (1930).
(177) Ludwig, W., and Mutzenbecher, P.: Z. physiol. Chem. **258**, 210 (1939).
(178) Lüttringhaus, A.: Ann. **528**, 223 (1937).
(179) Lüttringhaus, A., and Säáf, G. v.: Ann. **542**, 241 (1939).
(180) McCombie, H., MacMillan, W. G., and Scarborough, H. A.: J. Chem. Soc. **1930**, 1202.
(181) McCombie, H., MacMillan, W. G., and Scarborough, H. A.: J. Chem. Soc. **1931**, 529.
(182) Maclay, W. D., and Hamilton, C. S.: J. Am. Chem. Soc. **54**, 3310 (1932).
(183) Maeyer, F., and Krieger, W.: Ber. **55**, 1659 (1922).
(184) Maikopar: Ber. **6**, 564 (1873).
(185) Mailhe, A., and Moureu, M. C.: Compt. rend. **154**, 1240 (1912).
(186) Mailhe, A., and Murat, M.: Compt. rend. **154**, 601 (1912).
(187) Mailhe, A., and Murat, M.: Bull. soc. chim. [4] **11**, 288 (1912).
(188) Marty, A.: Compt. rend. **187**, 47 (1928).
(189) Matsumura, K.: J. Am. Chem. Soc. **52**, 3201 (1930).
(190) Mecke, R.: Z. physik. Chem. **261**, 253 (1939).
(191) Merz, V., and Weith, W.: Ber. **14**, 187 (1881).
(192) Meyer, K. H., and Bergius, F.: Ber. **47**, 3158 (1914).
(193) Meyer, H., and Hofman, A.: Monatsh. **37**, 705 (1916).
(194) Moldavski, B. L., and Livshitz, S.: J. Gen. Chem. (U.S.S.R.) **4**, 948 (1934).
(195) Mole, J. D. C., and Turner, E. E.: J. Chem. Soc. **1939**, 1720.
(196) Müller, E., and Bunge, W.: Ber. **69**, 2164 (1936).
(197) Mutzenbecher, P. v.: Z. physiol. Chem. **261**, 253 (1939).
(198) Nakai, R.: Bull. Chem. Soc. Japan **5**, 136 (1930).
(199) Niemann, C., Benson, A. A., and Mead, J. F.: J. Am. Chem. Soc. **63**, 2204 (1941).
(200) Niemann, C., and Mead, J. F.: J. Am. Chem. Soc. **63**, 2685 (1941).
(201) Niemann, C., Mead, J. F., and Benson, A. A.: J. Am. Chem. Soc. **63**, 609 (1941).
(202) Niemann, C., and Redemann, C. E.: J. Am. Chem. Soc. **63**, 1549 (1941).
(203) Nietzki, R., and Schündelen, B.: Ber. **24**, 3586 (1891).
(204) Nollau, E. H., and Daniels, L. C.: J. Am. Chem. Soc. **36**, 1885 (1914).
(205) Norris, J. F., MacIntire, B. G., and Corse, W. M.: Am. Chem. J. **29**, 127 (1903).
(206) Ogawa, T.: Bull. Chem. Soc. Japan **6**, 174 (1931).
(207) Österlin, M.: Monatsh. **57**, 31 (1931).
(208) Paal, C., and Deybeck, S.: Ber. **30**, 884 (1897).
(209) Pearson, J. H., and Hammaren, B. W.: U. S. patent 2,349,459 (May 23, 1944).
(210) Peratoner, A., and Ortoleva, G.: Gazz. chim. ital. **28**, 197 (1898).
(211) Postowsky, J., and Lugowkin, B.: J. prakt. Chem. [2] **122**, 141 (1929).
(212) Prey, V.: Ber. **76B**, 156 (1943).
(213) Pummerer, R., Melamed, D., and Puttfarcken, H.: Ber. **55B**, 3116 (1922).
(214) Purgotti, A.: Gazz. chim. ital. **44**, I, 643 (1914).
(215) Pützer, B., and Schönhöfer, F.: German patent 550,327 (October 24, 1932).
(216) Quilico, A.: Atti accad. Lincei [6] **6**, 512 (1927).
(217) Raiford, L. C., and Colbert, J. C.: J. Am. Chem. Soc. **48**, 2652 (1926).
(218) Raiford, L. C., Thiessen, G. W., and Wernert, I. J.: J. Am. Chem. Soc. **52**, 1205 (1930).
(219) Rarick, M. J., Brewster, R. Q., and Dains, F. B.: J. Am. Chem. Soc. **55**, 1289 (1933).
(220) Reilly, J., and Barrett, H. S. B.: J. Chem. Soc. **1927**, 1399.
(221) Reilly, J., and Drumm, P. J.: J. Chem. Soc. **1927**, 2814.
(222) Reilly, J., and Drumm, P. J.: J. Chem. Soc. **1930**, 455.
(223) Reilly, J., Drumm, P. J., and Barrett, H. S. B.: J. Chem. Soc. **1927**, 67.
(224) Reilly, J., Drumm, P. J., and Gray, T.: Sci. Proc. Roy. Dublin Soc. **19**, 461 (1930).

(225) Rittler, K. W.: German patent 588,650 (November 23, 1933).

(226) Rittler, K. W.: German patent 609,080 (February 8, 1935).

(227) Rittler, K. W., and Rost, A.: German patent 611,400 (March 7, 1935).

(228) Roberts, E., and Turner, E. E.: J. Chem. Soc. **127,** 2004 (1925).

(229) Roberts, K. C., and Clark H. B.: J. Chem. Soc. **1935,** 1312.

(230) Roberts, K. C., and de Worms, C. G. M.: J. Chem. Soc. **1934,** 727.

(231) Roberts, K. C., and de Worms, C. G. M.: J. Chem. Soc. **1935,** 1309.

(232) Roberts, K. C., de Worms, C. G. M., and Clark, H. B.: J. Chem. Soc. **1935,** 196.

(233) Robinson, R., and Sugasawa, S.: J. Chem. Soc. **1931,** 3173.

(234) Ryan, H., Keane, J., and M'Gahon, J. C.: Proc. Roy. Irish Acad. **37B,** 368 (1927).

(235) Sabatier, P., and Mailhe, A.: Compt. rend. **151,** 493 (1910).

(236) Sabatier, P., and Mailhe, A.: Compt. rend. **155,** 261 (1912).

(237) Sabatier, P., and Mailhe, A.: Bull. soc. chim. [4] **11,** 843 (1912).

(238) Sabatier, P., and Mailhe, A.: Compt. rend. **158,** 611 (1914).

(239) Santos, A. C.: Dissertation Westfälischen Wilhelms-Universität, Münster, 1929, p. 5.

(240) Sartoretto, P. A., and Sowa, F. J.: J. Am. Chem. Soc. **59,** 603 (1937).

(241) Saunders, K. H.: *The Aromatic Diazocompounds*, p. 33. Edward Arnold and Co., London (1936).

(242) Scarborough, H. A.: J. Chem. Soc. **1929,** 2361.

(243) Scarborough, H. A., and Sweeten, J. L.: J. Chem. Soc. **1934,** 52.

(244) Scarborough, H. A., and Sweeten, J. L.: J. Chem. Soc. **1934,** 867.

(245) Schickh, O. V.: Ber. **69,** 242 (1936).

(246) Schiemann, G., and Winkelmüller, W.: J. prakt. Chem. **135,** 101 (1932).

(247) Schroeder, W. D., and Brewster, R. Q.: J. Am. Chem. Soc. **60,** 751 (1938).

(248) Schuegraf, K.: Helv. Chim. Acta **12,** 405 (1929).

(249) Shorygin, P., and Skoblinskaya, S. A.: Compt. rend. acad. sci. U. R. S. S. **14,** 505 (1937).

(250) Slotta, K. H., and Soremba, K. H.: Ber. **68,** 2059 (1935).

(251) Slotta, K. H., and Soremba, K. H.: Ber. **69,** 566 (1936).

(252) Smyth, C. P., and Walls, W. S.: J. Am. Chem. Soc. **54,** 3230 (1932).

(253) Späth, E.: Monatsh. **35,** 319 (1914).

(254) Späth, E., and Pickl, J.: Ber. **62,** 2251 (1929).

(255) Staudinger, H., and Staiger, F.: Ann. **517,** 67 (1935).

(256) Stöhr, R.: Z. physiol. Chem. **201,** 142 (1931).

(257) Stroud, S. W.: J. Endocrinol. **2,** 55 (1940).

(258) Suter, C. M.: J. Am. Chem. Soc. **51,** 2581 (1929).

(259) Suter, C. M.: J. Am. Chem. Soc. **53,** 1112 (1931).

(260) Suter, C. M., and Green, F. O.: J. Am. Chem. Soc. **59,** 2578 (1937).

(261) Suter, C. M., McKenzie, J. P., and Maxwell, C. E.: J. Am. Chem. Soc. **58,** 717 (1936).

(262) Suter, C. M., and Maxwell, C. E.: *Organic Syntheses*, Vol. 18, p. 64. John Wiley and Sons, Inc., New York (1938).

(263) Suter, C. M., and Oberg, E.: J. Am. Chem. Soc. **53,** 1567 (1931).

(264) Suter, C. M., and Scrutchfield, P. H.: J. Am. Chem. Soc. **58,** 54 (1936).

(265) Sutton, L. E., and Hampson, G. C.: Trans. Faraday Soc. **31,** 945 (1935).

(266) Thompson, M. C., and Turner, E. E.: J. Chem. Soc. **1938,** 29.

(267) Tomita, M.: J. Pharm. Soc. Japan **54,** 897 (1934).

(268) Tomita, M.: J. Pharm. Soc. Japan **56,** 492 (1936).

(269) Tomita, M.: J. Pharm. Soc. Japan **56,** 683 (1936).

(270) Tomita, M.: J. Pharm. Soc. Japan **56,** 814 (1936).

(271) Tomita, M.: J. Pharm. Soc. Japan **56,** 906 (1936).

(272) Tomita, M.: J. Pharm. Soc. Japan **57,** 689 (1937).

(273) Tomita, M.: J. Pharm. Soc. Japan **58,** 510 (1938).

(274) Tozer, B. H., and Smiles, S.: J. Chem. Soc. **1938,** 1897.

(275) Tozer, B. H., and Smiles, S.: J. Chem. Soc. **1938,** 2052.
(276) Turner, E. E., and Sheppard, A. B.: J. Chem. Soc. **127,** 544 (1925).
(277) Ullmann, F.: Ber. **29,** 1878 (1896).
(278) Ullmann, F.: Ber. **37,** 854 (1904).
(279) Ullmann, F., and Eiser, O.: Ber. **49,** 2162 (1916).
(280) Ullmann, F., and Engi, G.: Ber. **37,** 2368 (1904).
(281) Ullmann, F., Engi, G., and Herre, E.: Ann. **366,** 86 (1909).
(282) Ullmann, F., and Kipper, H.: Ber. **38,** 2122 (1905).
(283) Ullmann, F., and Panchaud, L.: Ann. **350,** 113 (1906).
(284) Ullmann, F., and Sponagel, P.: Ber. **38,** 2212 (1905).
(285) Ullmann, F., and Sponagel, P.: Ann. **350,** 87 (1906).
(286) Ullmann, F., and Stein, A.: Ber. **39,** 623 (1906).
(287) Ullmann, F., and Wagner, C.: Ann. **355,** 360 (1907).
(288) Ullmann, F., and Wagner, C.: Ann. **371,** 388 (1909).
(289) Ullmann, F., and Slokasoff, M.: Ber. **38,** 2118 (1905).
(290) Ullock, D. S., Gaffert, G. A., Konz, P. R., and Brown, G. G.: Trans. Am. Inst. Chem. Engrs. **32,** 73 (1936).
(291) Underwood, H. W., Baril, O. L., and Toone, G. C.: J. Am. Chem. Soc. **52,** 4087 (1930).
(292) Ungnade, H. E.: J. Am. Chem. Soc. **63,** 2091 (1941).
(293) Ungnade, H. E., and Orwoll, E. F.: J. Am. Chem. Soc. **65,** 1736 (1943).
(294) Walker, J.: J. Chem. Soc. **1942,** 347 (231).
(295) Walter, M.: Festschrift Emil C. Barell **1936,** 266; Chem. Zentr. **1937,** II, 380.
(296) Warren, L. A., and Smiles, S.: J. Chem. Soc. **1930,** 1327.
(296a) Warren, L. A., and Smiles, S.: J. Chem. Soc. **1930,** 962.
(297) Warren, L. A., and Smiles, S.: J. Chem. Soc. **1931,** 914.
(298) Warren, L. A., and Smiles, S.: J. Chem. Soc. **1931,** 2207.
(299) Warren, L. A., and Smiles, S.: J. Chem. Soc. **1932,** 1040.
(300) Weber, F. C., and Sowa, F. J.: J. Am. Chem. Soc. **60,** 94 (1938).
(301) Weston, P. E., and Adkins, H.: J. Am. Chem. Soc. **50,** 859 (1928).
(302) Willgerodt, C.: Ber. **12,** 1277 (1879).
(303) Willgerodt, C.: Ber. **13,** 887 (1880).
(304) Willgerodt, C., and Hüetlin, E.: Ber. **17,** 1764 (1886).
(305) Willgerodt, C., and Kornblum, A.: J. prakt. Chem. [2] **39,** 294 (1889).

AROMATIC CYCLODEHYDRATION

CHARLES K. BRADSHER

Department of Chemistry, Duke University, Durham, North Carolina

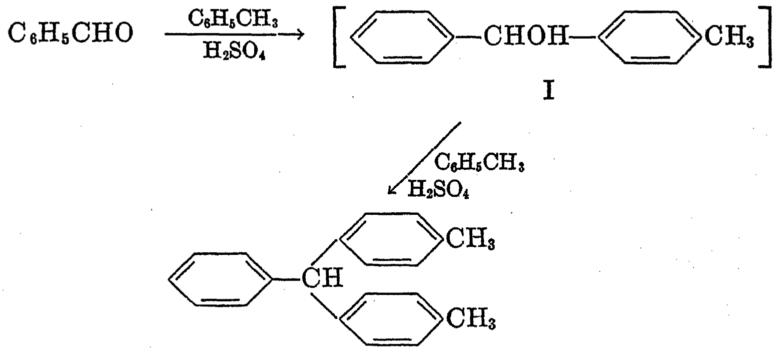
Received August 27, 1945

CONTENTS

I. Introduction 447
II. Bicyclic systems 449
    A. Naphthalene system 449
    B. Benzofuran system 455
    C. Thianaphthene system 458
    D. Indole system 459
    E. Quinoline system 463
    F. Isoquinoline system 469
III. Tricyclic systems 471
    A. Phenanthrene system 471
    B. Anthracene system 477
    C. Acridine system 479
IV. Mechanism 487
    A. General mechanism 487
    B. Phenanthrene system 488
    C. Anthracene system 492
V. Summary 495
VI. References 496

I. INTRODUCTION

It is well known that the carbonyl groups of aldehydes and, much more rarely, ketones in the presence of acid catalysts may condense with reactive aromatic nuclei. If the condensation is intermolecular in character, the carbonyl oxygen is usually replaced by two such nuclei. Evidently, the first step in this reaction is, in effect, the addition of an aromatic nucleus to a carbonyl linkage and is followed by further reaction with the resulting carbinol. This may be exemplified by the condensation of benzaldehyde with toluene in the presence of sulfuric acid to yield di(*p*-tolyl)phenylmethane (II) (101), the probable intermediate being 4-methylbenzohydrol (I).

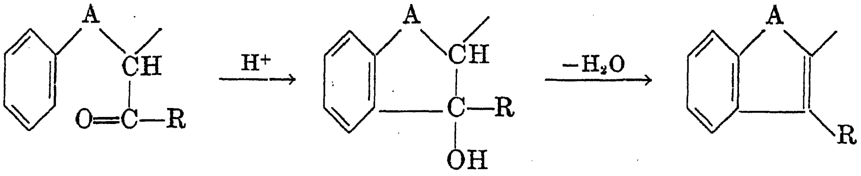


I

II

If the condensation is intramolecular, as illustrated by the general equation below, the cyclic carbinol (IV) first formed usually undergoes dehydration with

General equation



III IV V

(R = alkyl, aryl, or H; A = —C=C—, —O—, —S—, —NH—, —N=C—, —C=N—)

the establishment of a new double bond. If the other component of the new ring is such that it will not stabilize this double bond (e.g., V, A = —CH_2—), the newly formed compound will be prone to undergo further change in the presence of the cyclizing agent. If, on the other hand, the other component is capable of stabilizing the newly created double bond by resonance (e.g., V, A = —CH=CH—), to that extent the new compound will be resistant to further attack and can be said to have aromatic character. This second class of cyclization has been designated as aromatic cyclodehydration (44) and forms the subject of the present review.

In confining this discussion to this second class of cyclizations, we do not seek to imply any difference in mechanism, but rather to correlate and evaluate a general type of cyclization which, in carbocyclic systems at least, is almost unique in its ability to afford a new, fully aromatic ring without resort to dehydrogenation.

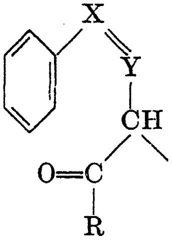
While cyclodehydrations of the aromatic type were applied in the synthesis of many heterocyclic derivatives as early as the end of the last century, it was not until 1935 that its systematic application in the carbocyclic system was initiated by Haworth and Sheldrick (87), working in the naphthalene series. Its use in the phenanthrene (38) and anthracene (11, 27) series is an even more recent development.

This review has been prepared in the hope that correlation and evaluation of the known examples of aromatic cyclodehydration may point the way to further applications of this interesting type of reaction.

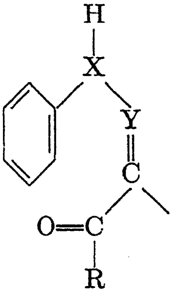
The cyclizations to be considered have been divided into bicyclic and tricyclic, depending on the nature of the final product. Heterocyclic systems have been arranged to follow their carbocyclic counterparts.

Although it has been stated that all of the cyclization products which will be considered possess aromatic character, some are much less resistant to the attack of acid than are others. An example is benzofuran which, because of its tendency to polymerize in acid solution, corresponds more nearly to indene than to naphthalene.

In those cases in which the new ring is to have six members, it is not necessary that the original double bond have the position shown in formula VI. It may be conjugated instead with the carbonyl group (VII). This latter is undoubtedly the structure of the intermediate in the anthracene and acridine syntheses and may be as well that involved in the quinoline synthesis. Under such circumstances, the dehydration of the carbinol intermediate is transannullar in character, but in no other way does it differ essentially from the general reaction already discussed. A more complete treatment of this aspect will be found in the section on mechanism.



VI

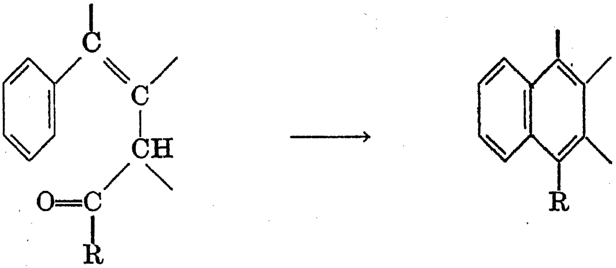


VII

II. BICYCLIC SYSTEMS

A. Naphthalene system

From the general equation it might be predicted that the cyclization of β-styrylacetaldehyde or β-styrylmethyl ketones (I, R = H, alkyl, or aryl) would yield naphthalene derivatives (II). In actual practice, it will be found that rather severe limitations are put on this cyclization by the presence of a double bond as well as a reactive methylene group, both of which may be affected under conditions employed in the cyclization. An additional complication rising out of the presence of the double bond is the possibility of geometric isomerism, an important factor when only one of the two forms could be expected

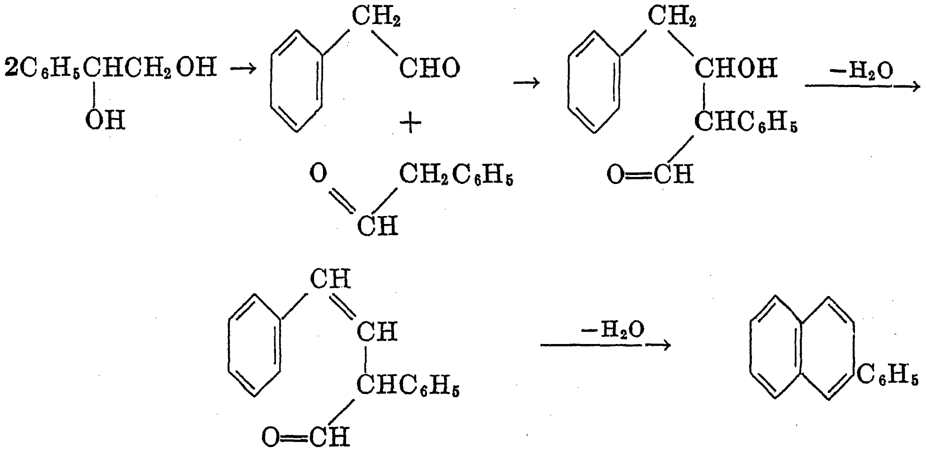


I II

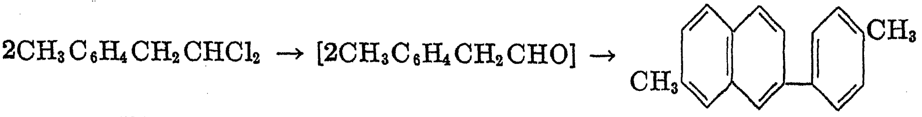
(R = H, alkyl, or aryl)

to cyclize. Despite these limitations, several instances of the naphthalene type of cyclization have been observed.

Perhaps the first case of aromatic cyclodehydration was encountered in 1884, when Zincke and Breuer (153) observed that phenylglycol or phenylacetaldehyde, in the presence of boiling 50 per cent sulfuric acid, yielded a hydrocarbon which was shown (154) to be β-phenylnaphthalene. Zincke assumed, in the case of the phenylethylene glycol, that the reactions involved are dehydration to phenylacetaldehyde followed by aldol condensation of two molecules of phenylacetaldehyde, dehydration, and finally cyclization.

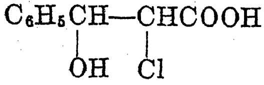


Similarly, a homolog (IV) of β-phenylnaphthalene was produced by von Auwers and Keil (2, 3) by the hydrolysis of *p*-methyl(β,β-dichloroethyl)benzene (III).



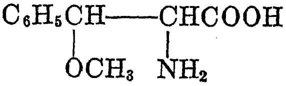
III IV

The formation of β-phenylnaphthalene appears characteristic of all compounds which yield phenylacetaldehyde when refluxed with boiling mineral acids. These include, in addition to phenylethylene glycol, β-phenyl-β-hydroxyethylamine (55), α-chloro-β-hydroxy-β-phenylpropionic acid (V) (3), phenylserine (20a) and its methyl ether (VI) (55). Späth (134) proposed that the conversion of the γ-lactone (VII) of α-hydroxy-β-phenyl-γ-hydroxy-δ-phenyl-



$$C_6H_5\underset{OH}{CH}-\underset{Cl}{C}HCOOH$$

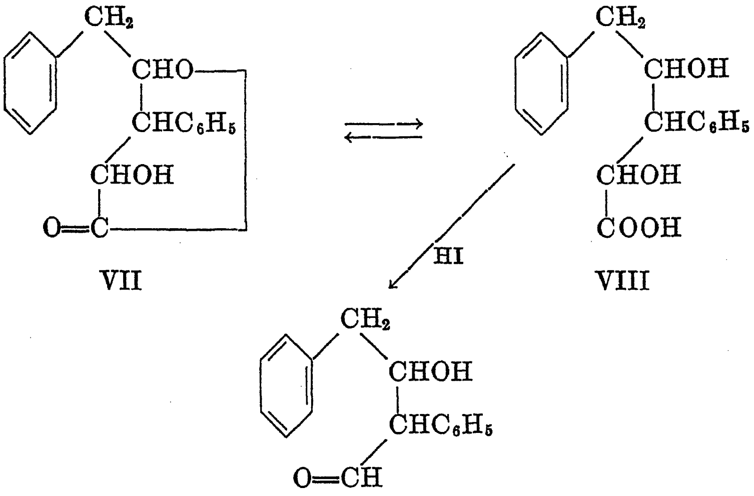
V



$$C_6H_5\underset{OCH_3}{CH}-\underset{NH_2}{C}HCOOH$$

VI

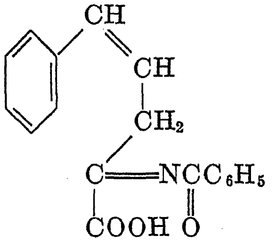
valeric acid to β-phenylnaphthalene might likewise involve fission into molecules of phenylacetaldehyde, followed by recombination.



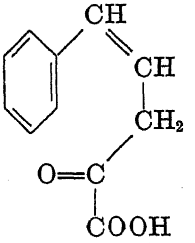
VII VIII IX

An equally plausible explanation is that the α-hydroxy acid (VIII) merely loses carbon monoxide and water, yielding the hydroxyaldehyde (IX) which is the first intermediate proposed in the Zincke mechanism for phenylacetaldehyde cyclization.

Erlenmeyer and coworkers (62, 63) observed that the α-benzimino-β-(β-styryl)propionic acid (X), upon hydrolysis with hydrochloric acid at 120°C., gave a mixture of naphthalene and α-naphthoic acid. These authors postulated (β-styryl)pyruvic acid (XI) as an intermediate.

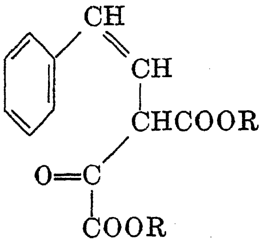


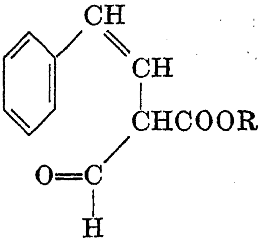
X



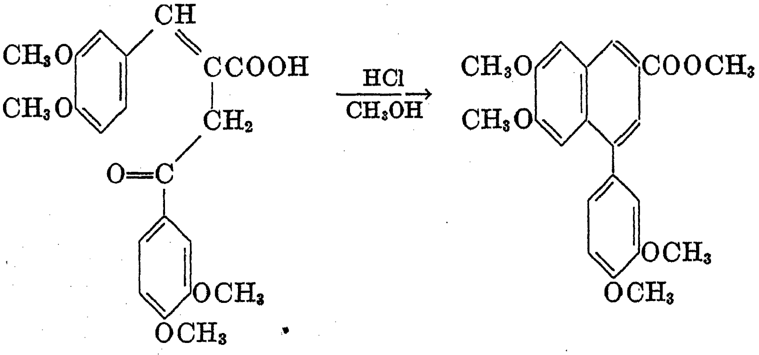
XI

The first recorded attempts to prepare a naphthalene derivative directly by cyclization of an unsaturated aldehyde or ketone were made by von Auwers and Möller (5). The condensation products of ethyl oxalate (XII) or ethyl formate (XIII) with ethyl (β-styryl)acetate resisted all attempts at cyclization using sulfuric acid or phosphorus pentoxide. von Auwers and Möller postulated that XII was in the *trans* configuration and showed that XIII polymerized rapidly under the conditions used in the attempted cyclization.


XII


XIII

It remained for Haworth and his students to provide the first clear-cut case of the cyclization of a β-styryl ketone and to make important application of this reaction to the synthesis of phenolic resin derivatives. Haworth and Sheldrick (87) observed that when β-3,4-dimethoxybenzoyl-α-(3′,4′-dimethoxybenzylidene)propionic acid (XIV) was refluxed for 4 hr. with a methyl alcohol solution of hydrogen chloride, the methyl ester (XV) of 6,7-dimethoxy-1-(3′,4′-dimethoxyphenyl)naphthalene-3-carboxylic acid was obtained. When the lactone of XIV was allowed to stand in a chloroform solution containing iodine, the acid corresponding to XV was obtained.


XIV XV

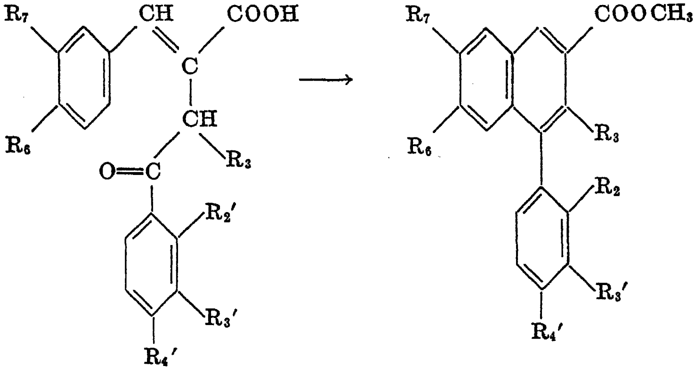
This type of reaction was reported a little later by Howell and Robertson (92) and finally, Ohmaki (119) made a thorough study of the effect of substituents on the ease of cyclization in such systems. A summary of the results of all these authors is to be found in table 1.

Ohmaki concluded that there must be a hydroxyl or alkoxyl group para to the point at which cyclization is to take place or, in other words, R_7 must be a hydroxyl, alkoxyl, or a terminus of a methylenedioxy group. The ease of cyclization did not appear to be influenced by the nature of the substituents ($R_{2'}$, $R_{3'}$, and $R_{4'}$) in the ring attached to the carbonyl group.

Of the groups capable of activating cyclization, the hydroxyl ($R_7 = OH$) was the most potent. The α-(*m*-hydroxybenzal)-β-anisylpropionic acid (XVII) as a consequence could not be prepared from its lactone (XVI), for cyclization took place spontaneously, yielding the α-phenylnaphthalene derivative (XVIII).

TABLE 1

Cyclization experiments with α-benzal-β-benzoylpropionic acids



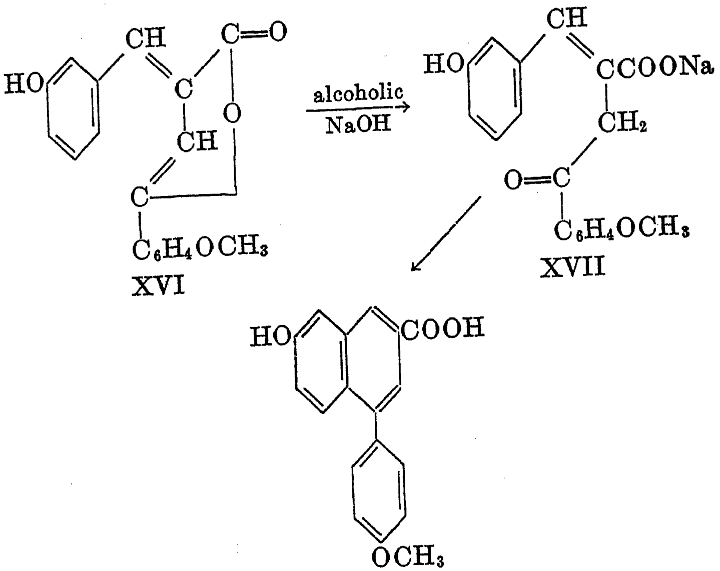
Cyclized

R_3	R_6	R_7	R_2'	R_3'	R_4'	REFERENCES
H	OCH_3	OCH_3	H	H	H	(92, 119)
H	—O—CH_2—O—		H	H	H	(119)
H	OH	OCH_3	H	H	OCH_3	(119)
H	—O—CH_2—O—		H	H	OCH_3	(119)
H	H	OH	H	H	OCH_3	(119)
H	OH	OCH_3	H	OCH_3	OCH_3	(119)
H	OCH_3	OCH_3	H	OCH_3	OCH_3	(87, 119)
CH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	(88)
H	OC_2H_5	OCH_3	H	OCH_3	OCH_3	(119)
H	—O—CH_2—O—		H	OCH_3	OCH_3	(119)
H	OH	OCH_3	OCH_3	H	OCH_3	(119)
H	—O—CH_2—O—		OCH_3	H	OCH_3	(119)

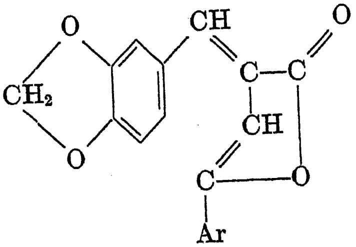
Failed to cyclize
(R_3 and R_2' = H)

R_6	R_7	R_3'	R_4'	REFERENCES
H	H	H	H	(119)
H	H	H	CH_3O	(92, 119)
CH_3O	H	H	CH_3O	(119)
H	H	CH_3O	CH_3O	(119)
CH_3O	H	CH_3O	CH_3O	(119)
CH_3	H	CH_3O	CH_3O	(119)
H	CH_3	CH_3O	CH_3O	(119)

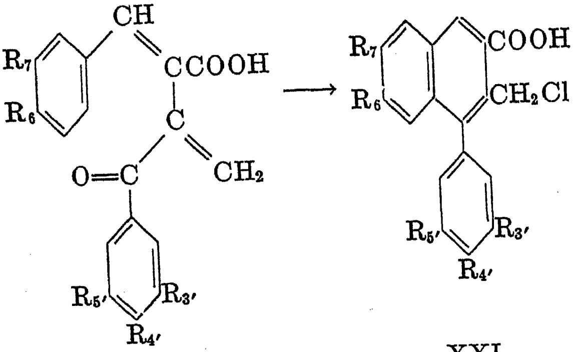
After the hydroxyl, the alkoxyl groups (R_7 = OR) appear to stand next in activating influence, with yields of the order of 85 per cent being reported (87).



XVI XVII XVIII



XIX



XX XXI

($R_{3'}$ = H; R_6, R_7, $R_{4'}$, $R_{5'}$ = OCH_3)

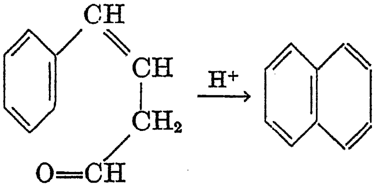
XXII XXIII

($R_{3'}$, $R_{4'}$, $R_{5'}$ = OCH_3 ; R_6—R_7 = —O—CH_2—O—)

The methylenedioxy group at the same position (R_6 and R_7 = —O—CH$_2$—O—) is reported to give the poorest results, the yields after 8–15 hr. refluxing being not over 5 to 8 per cent. Ohmaki attributes this to the intermediate formation of the difficultly soluble lactone (XIX).

One other reaction which may be considered in this connection was observed by Haworth, Richardson, and Sheldrick (86), who found that β-3,4-dimethoxybenzoyl-α-(3′,4′-dimethoxybenzylidene)-β-methylenepropionic acid (XX) undergoes cyclization and addition of hydrogen chloride to yield 6,7-dimethoxy-1-(3,4-dimethoxyphenyl)-2-chloromethylnaphthalene-3-carboxylic acid (XXI). This reaction was further extended (XXII→XXIII).

The simplest example of aromatic cyclodehydration in the carbocyclic series and, indeed, one which might be considered the prototype, is the cyclization of β-styrylacetaldehyde (XXIV) to yield naphthalene. The aldehyde was first synthesized by Rinkes (128), who reported that he had certain proof that



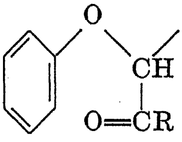
XXIV

it could be converted to naphthalene, although the amount of aldehyde available precluded an investigation of this problem.

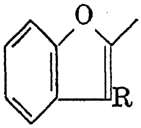
In order to determine whether the cyclization was an acid-catalyzed reaction of the general type under consideration, the author (29) prepared the aldehyde (XXIV) and found that refluxing with hydrobromic and acetic acids gave naphthalene in a yield of 25 per cent.

B. Benzofuran system

The formation of the benzofuran nucleus (XXVI) by aromatic cyclodehydration requires, as a starting material, a phenoxyacetaldehyde or phenoxymethyl ketone (XXV, R = H, alkyl, or aryl). Having no double bond, the aldehyde or ketone is more stable toward acid than its counterpart in the naphthalene system and there is no problem arising from geometric isomerism. The presence of a strong *o*–*p*-directing ether linkage results in a markedly greater ease of cyclization. This is an important feature, in that the stability of the ben-



XXV

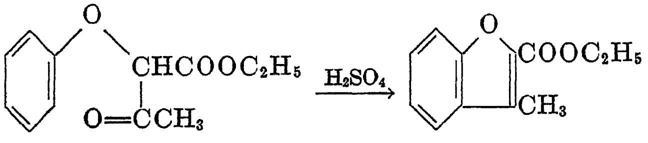


XXVI

(R = H, alkyl, or aryl)

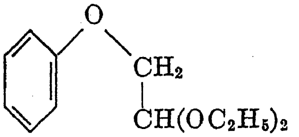
zofuran nucleus (XXVI) in acidic media is considerably less than that shown by naphthalene derivatives.

The first application of such a cyclodehydration to the preparation of benzofuran derivatives was made in 1886 by Hantzsch (84, 85), who found that the material produced by condensation of sodium phenoxide with α-chloroacetoacetic ester (presumably α-phenoxyacetoacetic ester (XXVII)) would undergo cyclization in the presence of concentrated sulfuric acid to yield 2-carbethoxy-3-methylbenzofuran (XXVIII). The reaction was extended (85) to the corresponding naphthofurans by the use of α- and β-naphthoxyacetoacetic esters.

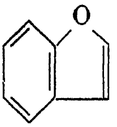


XXVII XXVIII

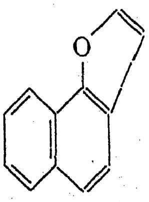
A few years later, in a paper dealing mainly with sulfur compounds, Autenrieth (1) described the preparation of phenoxyacetal (XXIX) and, citing the analogy to the results of Hantzsch, predicted that it could be cyclized to benzofuran (XXX). His paper gives no indication that this cyclization was actually carried out. Pomeranz (126) reported that all of his attempts to cyclize phenoxyacetal resulted in failure, owing to the easy polymerization of benzofuran in the presence of mineral acids. Hesse (91) likewise failed to cyclize phenoxy- or cresyloxy-acetal, but did succeed in preparing the corresponding α- and β-naphthofurans (XXXI and XXXII) by heating together at 200°C. alcoholic solutions of potassium hydroxide with chloroacetal and α- or β-naphthol.



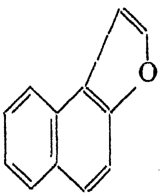
XXIX



XXX



XXXI



XXXII

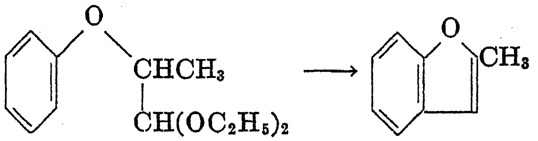
It is significant that when sodium ethoxide in alcohol was used under the same conditions, no cyclization took place, but formation of naphthoxyacetal was observed.

$$C_{10}H_7OH + C_2H_5ONa + ClCH_2CH(OC_2H_5)_2 \rightarrow C_{10}H_7OCH_2CH(OC_2H_5)_2 + NaCl + C_2H_5OH$$

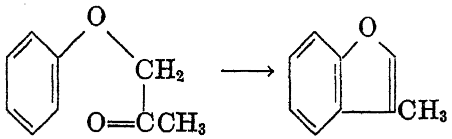
It seems probable that, in the cases where cyclization was observed, water acted as a source of protons for the cyclization.

From certain observations (137) which will be discussed later, Stoermer concluded that cyclization of phenoxy- and cresyloxy-acetals and acetaldehydes could be accomplished if a cyclizing agent of the proper strength could be found. He found that an acetic acid solution of zinc chloride functioned very well and was useful for the preparation of naphthofuran, and of benzofuran and its homologs. Owing to the great tendency of the products to undergo polymerization, yields rarely rose above 10 per cent. In the case of the acetal, a good reagent for the cyclization was found to be anhydrous oxalic acid.

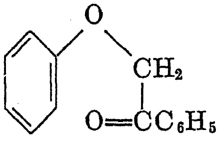
This type of cyclization is quite general in its nature. The acetal (XXXIII) of α-phenoxypropionaldehyde cyclizes to yield 1-methylbenzofuran (XXXIV) (138). Stoermer (137) found that the action of sulfuric acid on phenoxyacetone (XXXV) yielded the 2-methyl isomer (XXXVI). This reaction could not be extended to the cyclization of α-phenoxyacetophenone (XXXVII) (139).



XXXIII XXXIV



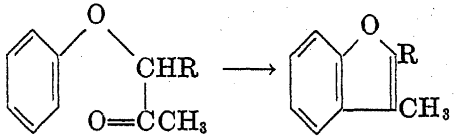
XXXV XXXVI



XXXVII

Benzofurans with two substituents in the five-membered ring (XXXIX) may be made by the use of suitably substituted ketones (XXXVIII) (140, 148).

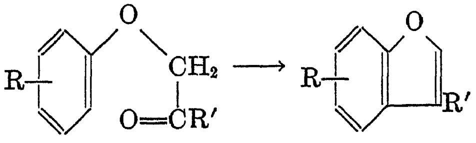
In addition, by varying the aryl groups, many derivatives can be made with



XXXVIII XXXIX

(R = CH_3, $CH_2C_6H_5$)

substituents in the six-membered ring (138). Stoermer (138) concluded that

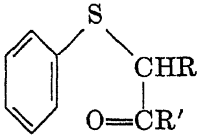


XL XLI

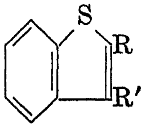
the presence of methyl groups in the aryl ring (XL) facilitated cyclization, while chlorine in the para-position (XL, R = *p*-Cl) greatly inhibited it. A nitro group in the para-position (XL, R = *p*-NO_2) appeared to prevent cyclization altogether.

C. Thianaphthene system

The similarity of the chemistry of sulfur to that of oxygen would suggest that α-phenylsulfhydryl ketones or aldehydes (XLII) might be cyclized to thianaphthene derivatives (XLIII). Such an idea occurred to the earliest workers in the benzofuran series. The almost universal lack of success experienced with the sulfur compounds seems to originate from the instability of the



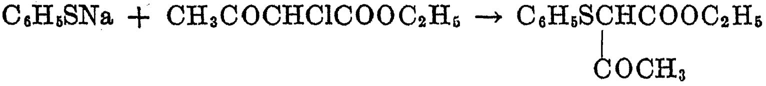
XLII



XLIII

phenylsulfhydryl ketones and aldehydes (XLII), as well as from a low order of ortho activation produced by the thioether group.

Hantzsch (84) attempted to prepare α-(phenylsulfhydryl)acetoacetic ester (XLIV) by the action of sodium phenylmercaptide with α-chloroacetoacetic ester in the hope that cyclization to 2-carbethoxy-3-methylthianaphthene (XLIII, R = $COOC_2H_5$, R′ = CH_3) might be accomplished. Unfortunately,

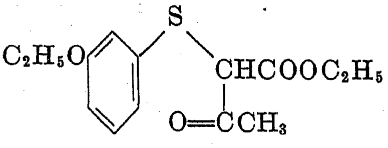


$$C_6H_5SNa + CH_3COCHClCOOC_2H_5 \rightarrow \underset{\displaystyle COCH_3}{C_6H_5S\underset{|}{C}HCOOC_2H_5}$$

XLIV

instead of the sulfhydryl ester, phenyl disulfide was obtained under these conditions.

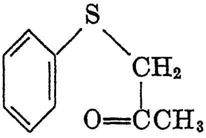
Delisle and Schwalm (61) succeeded in preparing phenylsulfacetoacetic ester (XLIV) and found that it decomposed on heating to yield phenyl disulfide and resisted all attempts at cyclization. The corresponding *m*-ethoxy derivative (XLV), which would be expected to be more likely to cyclize, was likewise

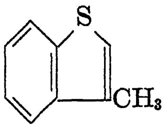


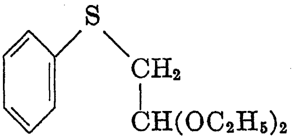
XLV

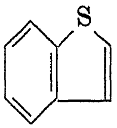
found to yield *m*-phenetole disulfide very readily, making attempts at cyclization equally futile.

In an earlier paper, Delisle (60) reported the synthesis of phenyl acetonyl sulfide (XLVI) and projected its cyclization to the corresponding 2-methylthianaphthene (XLVII), although no evidence was given that such a cyclization was carried out. Essentially the same idea was reported by Autenrieth (1), with no statement that the cyclization experiment had met with any success. Autenrieth likewise prepared thiophenylacetal (XLVIII), but was unable to effect its cyclization to thianaphthene (XLIX), although a variety of acidic cyclizing agents were tried.

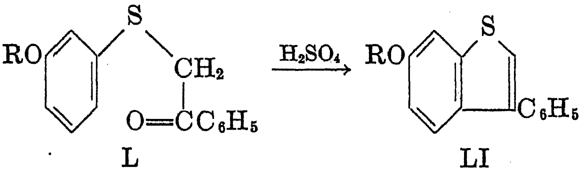

XLVI


XLVII


XLVIII


XLIX

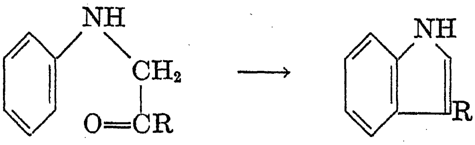
Apparently the only clear-cut case of a cyclization of this type in the thianaphthene series is afforded by the work of Fries and his colleagues (77), who observed that phenacyl 3-hydroxyphenyl sulfide (L, R = H) could be cyclized by concentrated sulfuric acid to yield 3-phenyl-6-hydroxythianaphthene (LI, R = H) in 45 per cent yield. A better yield was obtained using the methyl ether. Investigation of similar activated systems would appear desirable.



(R = H or CH_3)

D. *Indole system*

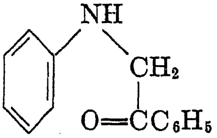
From the cyclizations already considered, it might be predicted that α-anilino ketones or aldehydes (LII) could be cyclized by acidic reagents to yield



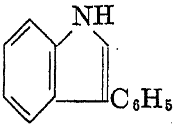
indoles (LIII). Actually, this mode of reaction appears to be more nearly the exception than the rule, cyclization usually being accompanied by real or apparent rearrangement.

The first ketone of this class to receive any extensive study was phenacylaniline (LIV), which Möhlau made by the reaction of phenacyl bromide with aniline (113). This phenylamino ketone, upon refluxing with aniline (113), dry distillation (115), or heating its hydrochloride with phosphorus pentachloride (115), yielded a new compound, m.p. 185°C. On the basis of a molecular-weight determination (114), later admitted to be erroneous (116), Möhlau concluded that the molecular formula was $C_{28}H_{22}N_2$, and the structure could be most adequately described as "diphenylpseudoamphiphenacylnitrile".

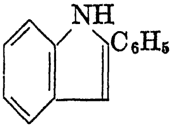
A few years later, Wolff (149) expressed the opinion that the dehydration product obtained by Möhlau was 3-phenylindole (LV). It remained for Fischer and Schmitt (71) to demonstrate that the product was in reality the isomeric 2-phenylindole (LVI), a compound whose structure had been well established by both synthesis (69, 122) and degradation (123).



LIV



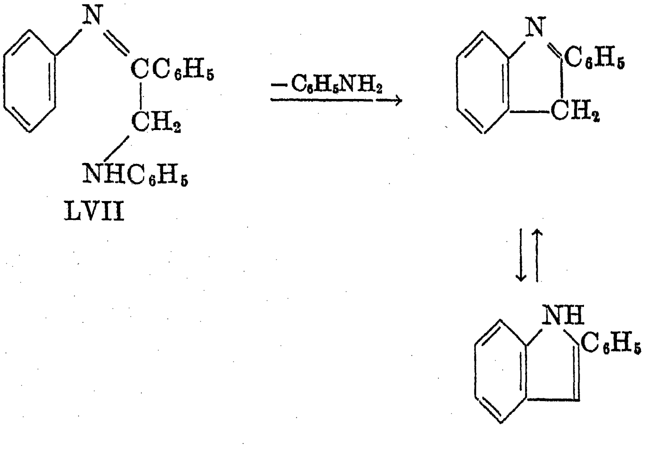
LV



LVI

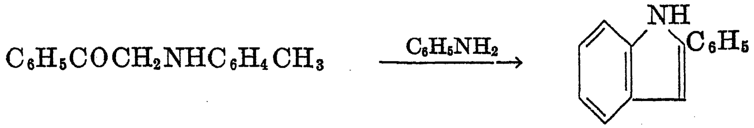
While Fischer and Schmitt (72) observed that 3-phenylindole will rearrange to the 2-isomer when heated with zinc chloride, Ince (93), by heating the 3-indole in the presence of aniline hydrochloride at temperatures as high as 230°C., demonstrated fairly well that rearrangement does not occur under the conditions usually used in the indole cyclization.

To this apparent dilemma, Bischler (22) offered an ingenious solution. According to his postulate, the phenacylaniline, first formed in the reaction between phenacyl bromide and aniline, reacts further to yield an anil (LVII) which cyclizes by loss of a molecule of aniline to yield 2-phenylindole. It is the phenyl



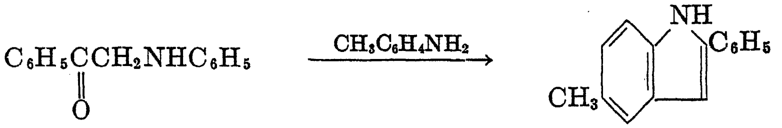
LVII

radical of the phenylimino rather than the phenylamino group which becomes part of the indole nucleus. Phenacylamines were refluxed with a variety of aromatic primary amines to demonstrate that the structure of the final indole was independent of the group originally associated with the phenacyl radical, and depended solely on the amine with which it had been refluxed. For example, phenacyl(*p*-toluide) (LVIII), when refluxed with aniline, gave 2-phenylindole,



LVIII

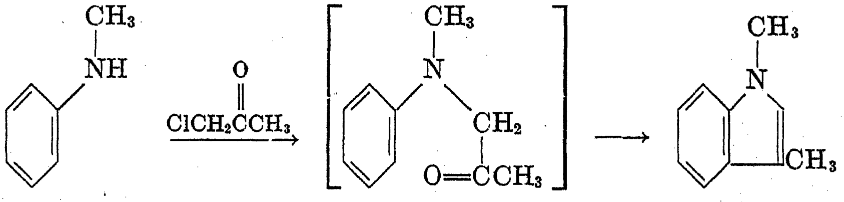
while phenacylaniline, when refluxed with *p*-toluidine, yielded 2-phenyl-5-methylindole (LIX).



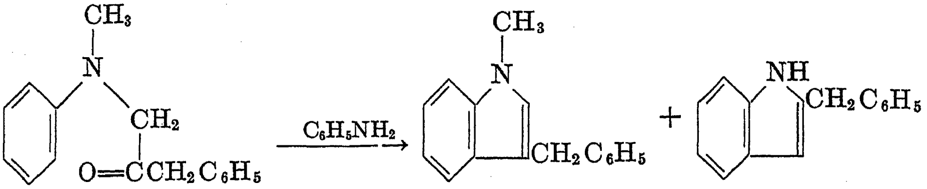
LIX

In a great many experiments (e.g., 23, 52, 58, 89, 90, 118, 135) the products actually obtained have been those predicted on the basis of the Bischler theory and it is not surprising that this theory has found almost universal acceptance.

In recent papers calling into question the validity of Bischler's assumption, Verkade and Janetsky (146, 147) have pointed out several examples of indole formation which could be explained on the basis of simple cyclodehydration. Nearly all of these cases involve or are believed to involve a phenylmethylamino ketone as an intermediate. Meisenheimer (111) observed that methylaniline with chloroacetone gave the 1,3-dimethylindole (LX) rather than the 1,2-isomer. Julian and Pikl (100) showed that when 1-phenylmethylamino-3-phenylpropanone-2 (LXI) was heated with aniline, in addition to the 2-benzylindole predicted by the Bischler theory, 1-methyl-3-benzylindole (LXII) was obtained. Verkade and Janetsky showed that both phenylmethylamino-

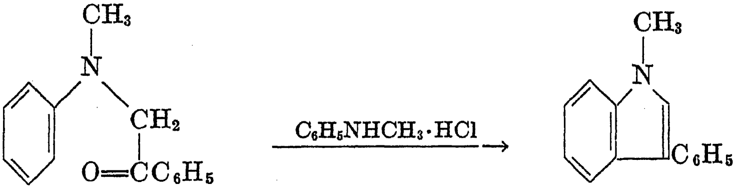


LX

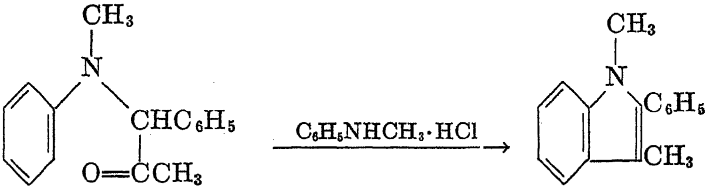


LXI LXII

acetophenone (LXIII) and 1-phenyl-1-phenylmethylaminopropanone-2 (LXV), when heated with *N*-methylaniline hydrochloride, yield the products which would be expected if simple cyclization had taken place,—namely, 1-methyl-3-phenylindole (LXIV) and 1,3-dimethyl-2-phenylindole (LXVI).

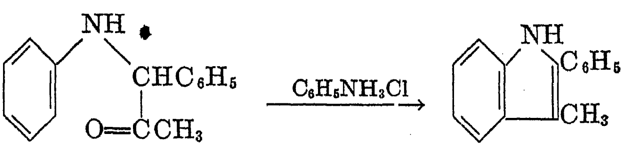


LXIII LXIV



LXV LXVI

While all of the cases considered above have had a methyl group attached to the nitrogen atom, Verkade and Janetsky have shown that indole formation by apparent cyclization is possible without this or any substituent. When 1-phenylamino-1-phenylpropanone-2 (LXVII) is heated with aniline hydrochloride, 2-phenyl-3-methylindole (LXVIII) is obtained in almost quantitative yield. Verkade and Janetsky have also shown that the formation of indoles from arylaminoketones is acid-catalyzed and does not occur in the absence of a catalyst. This confirms the earlier observations of Japp and Murray (95).



LXVII LXVIII

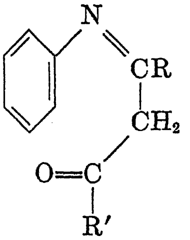
It may be safely concluded that in a few instances, α-arylaminoketones may undergo the general aromatic cyclodehydration reaction observed in other systems, but usually the reaction is more complicated and is as yet imperfectly understood.[1]

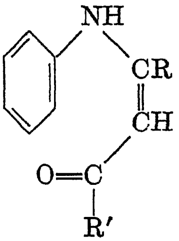
---

[1] Since this manuscript was completed, Julian, Meyer, Magnani, and Cole (J. Am. Chem. Soc. 67, 1203 (1945)) have published an excellent paper concerning the indole cyclization.

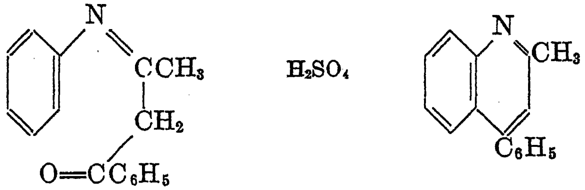
E. Quinoline system

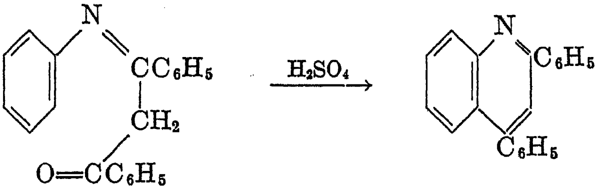
More readily comparable to the naphthalene than the heterocyclic systems thus far considered, quinoline may be prepared by the acid-catalyzed dehydration of diketone anils (LXIX or LXX). Whether the nitrogen is attached to the chain by an imine (LXIX) or a vinylamine linkage (LXX), it is known that the hydrolysis of the carbon-to-nitrogen bond tends to take place under the conditions used for cyclization. Despite this limitation, the ring closure of diketone anils, in particular those of acetylacetone (the Combes synthesis), constitutes one of the two most important and extensive applications of aromatic cyclodehydration.


LXIX


LXX

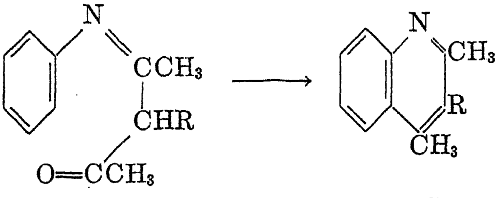
The first such cyclization was reported in 1887 by Beyer (21), who found that the anils of benzoylacetone (LXXI) and of dibenzoylmethane (LXXIII) may be cyclized by the action of concentrated sulfuric acid to yield 2-methyl-4-phenyl- and 2,4-diphenyl-quinolines (LXXII and LXXIV).


LXXI LXXII


LXXIII LXXIV

Much new evidence pertaining to the mechanism of cyclization has been presented, together with alternate explanations for some of the phenomena observed by Verkade and Janetsky. Julian and his coauthors have summarized as follows: "The experiments recorded in this paper justify on the whole the Bischler hypothesis with modifications. They do not rule out of consideration 'direct' ring closure with loss of water, and until more evidence is obtained we prefer to take the point of view that both reactions may take place, the evidence at present being still predominantly in favor of the Bischler hypothesis of intermediate diamine formation."[2]

The following year, Combes (53, 54) effected a similar cyclization, using the anil of acetylacetone (LXXV, R = H) to produce 2,4-dimethylquinoline (LXXVI, R = H). Similarly, methyl acetylacetone anil (LXXV, R = CH_3) yields 2,3,4-trimethylquinoline (LXXVI, R = CH_3).


LXXV LXXVI

As may be seen from table 2, the Combes synthesis has found many applications. Roberts and Turner (129) have made a thorough study of the factors influencing cyclization. The presence of a methoxyl group meta to the position at which cyclization is expected (R_6 or R_8 = OCH_3) completely inhibits the cyclization. An inhibition of this type has been observed in other cyclizations (66, 83, 94, 98, 112, 150) and is likewise quite characteristic of aromatic cyclodehydration (102, 103, 129, 142). The presence of a chlorine atom meta to the position of cyclization likewise prevents cyclization, but this may be in part due to the general deactivating influence of chlorine on the aromatic ring since the methyl group, which has about the same order of ortho–para-activating influence, shows no such inhibition.

In the case of the inhibition due to a *m*-methoxyl group, the deactivation can be overcome by introduction of a second methoxyl group ortho or para to the position at which cyclization is expected, as when R_7 and R_8 or R_4 and R_8 = OCH_3. In only one case was the same type of action observed in the corresponding dichloro compounds, the anil of 3,4-dichloroaniline (R_6 and R_7 = Cl) being converted, in a single experiment, to the 6,7-dichloroquinoline. A single ortho–para-directive group, ortho or para to the point at which cyclization is expected to occur, increases the ease of cyclization.

The Combes synthesis has been applied to the synthesis of benzoquinolines (54) starting from naphthylamines. The α-naphthylamine–acetylacetone condensation product (LXXVII) cyclizes readily to yield 2,4-dimethyl-7,8-benzoquinoline (LXXVIII). The anil (LXXIX) from β-naphthylamine gives

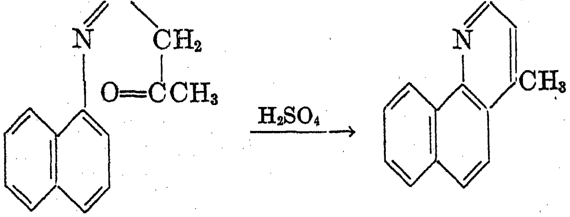
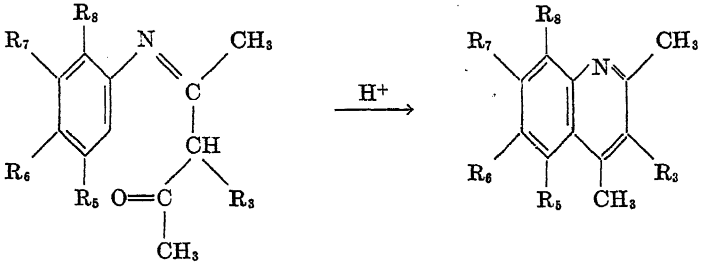

LXXVII LXXVIII

TABLE 2

Combes synthesis



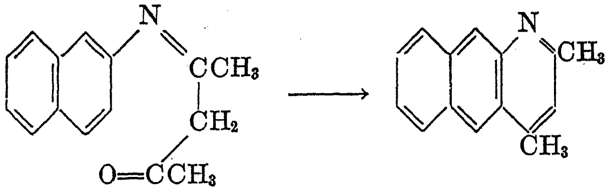
Cyclized

R_3	R_5	R_6	R_7	R_8	REFERENCES
H	H	H	H	H	(53, 54, 74)
CH_3	H	H	H	H	(53, 54)
H	H	CH_3	H	H	(53, 54)
H	H	C_2H_5	H	H	(82)
H	H	H	H	CH_3	(53, 54)
H	H	H	H	C_2H_5	(6)
H	H	H	H	n-C_3H_7	(8)
H	CH_3	H	H	CH_3	(47)
CH_3	H	H	H	CH_3	(7)
CH_3	H	H	H	C_2H_5	(81)
CH_3	H	H	H	n-C_3H_7	(132)
H	H	H	$—(CH_2)_4—$		(47)
H	H	H	OCH_3	OCH_3	(105a)
H	OCH_3	H	H	OCH_3	(105a)
H	H	OCH_3	OCH_3	OCH_3	(105)
H	OCH_3	OCH_3	OCH_3	H	(105)
H	OCH_3	OCH_3	H	Br	(105a)
H	H	H	Cl	H	(129)
H	H	Cl	Cl	H	(129)

Failed to cyclize
(R_3 = H)

R_5	R_6	R_7	R_8	REFERENCES
H	OCH_3	H	H	(103)
H	H	H	OCH_3	(129)
H	H	H	Cl	(129)
H	Cl	H	H	(129)
H	Cl	H	Cl	(129)
Cl	H	H	Cl	(129)
Cl	H	Cl	H	(129)

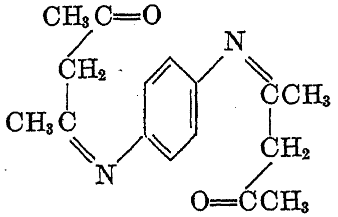
what has recently been shown (99) to be the 6,7-benzoquinoline analog (LXXX). The formation of the linear product is remarkable in that the condensation has



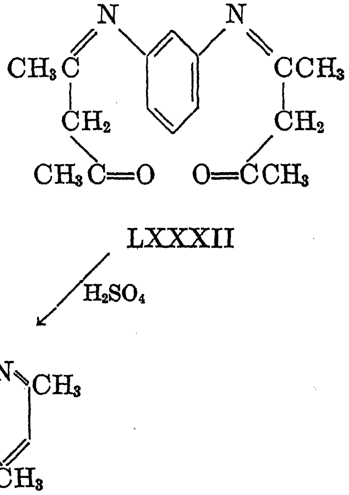
LXXIX LXXX

taken place at what is normally a relatively unreactive beta position, rather than the reactive alpha position.

Marckwald and Schmidt (106) first studied the reactions of the dianils from



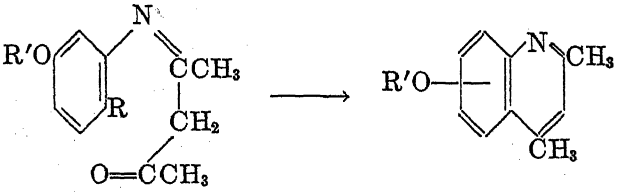
LXXXI



LXXXIII

p- and *m*-phenylenediamines. The dianil (LXXXI) from *p*-phenylenediamine simply underwent fission when treated with sulfuric acid, while its *m*-isomer (LXXXII), by a single cyclization and fission, gave what is believed (129) to be the 7-amino-2,4-dimethylquinoline (LXXXIII) rather than its 5-isomer.

The Combes synthesis has been used by Murray and Turner (117) to prepare some diquinolinyl ethers. The anils (LXXXIV) having groups ortho to the nitrogen atom (R = CH_3, OCH_3, OC_2H_5) could not be cyclized, while those



LXXXIV LXXXV

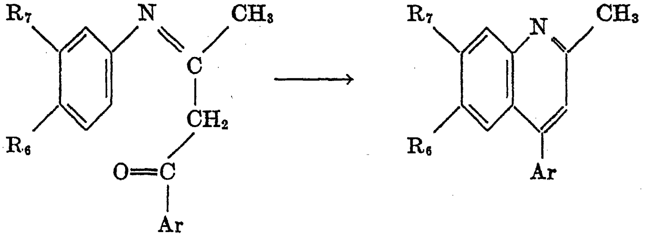
(R = H; R′ = 2-methyl-4-quinolinoxy or 4-methyl-2-quinolinoxy) having no such substituent (R = H) yielded the diquinolinyl ethers (LXXXV). The authors did not ascertain whether the quinolinoxy group was at the 7- or the 5-position of the new quinoline nucleus.

The somewhat less extensive data on the cyclization of the anils (LXXI) of benzoylacetone (table 3) suggests that the behavior of these compounds is very similar to that of the acetylacetone anils. In addition, it has been observed (103) that the presence of a methoxyl group meta to the point at which condensation is to be expected (R_6 = OCH_3) causes a complete inhibition of cyclization.

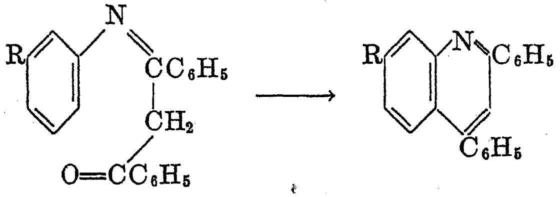
In his original observation, Beyer (21) found that the cyclization of the anil of dibenzoylmethane (LXXXVI, R = H) to 2,4-diphenylquinoline (LXXXVII, R = H) took place with great difficulty. So great is the activation produced by the introduction of a methoxyl group para to the point of expected cyclization, that cyclization under *these* circumstances may be accomplished in very good yield and under very mild conditions (104).

TABLE 3

Preparation of derivatives of 2-methyl-4-phenylquinoline



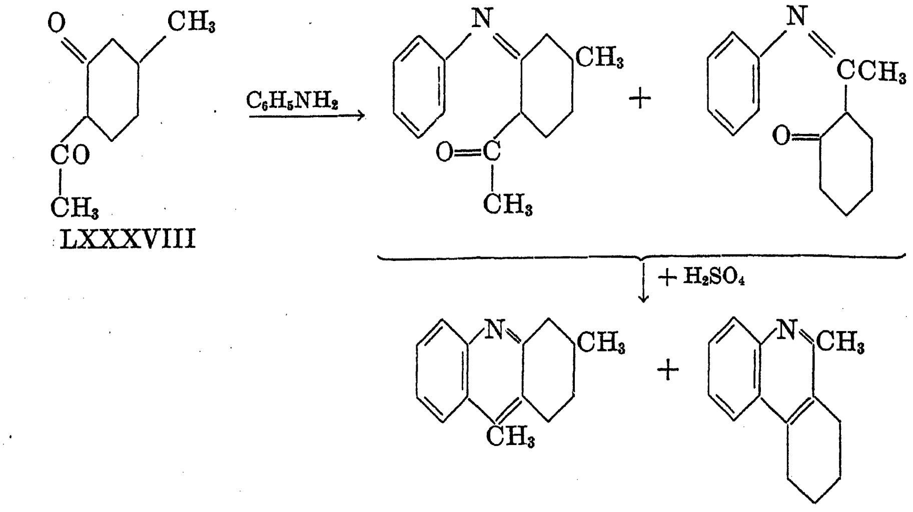
Ar	R_6	R_7	REFERENCES
C_6H_5	H	H	(21, 74)
C_6H_5	CH_3	H	(74)
o-$CH_3OC_6H_4$	H	H	(18, 20)
m-$CH_3OC_6H_4$	H	H	(18, 20)
p-$CH_3OC_6H_4$	H	H	(18, 20)
o-$C_2H_5OC_6H_4$	H	H	(18)
C_6H_5	H	OH	(49)



LXXXVI LXXXVII

(R = H, OCH_3)

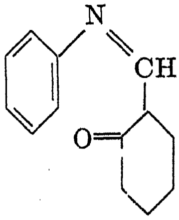
One other type of diketone anil which has been successfully cyclized is that of 5-methyl-2-acetylcyclohexanone (LXXXVIII).



LXXXVIII

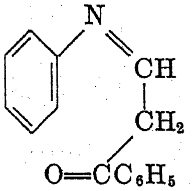
LXXXIX XC

While this can and does form an anil at either carbonyl, Borsche (24) found that conditions can be so controlled that most of the condensation takes place with the ring carbonyl. The two anils were not separated but were cyclized simultaneously by the action of sulfuric acid to yield a mixture which was 85 per cent 3,10-dimethyl-1,2,3,4-tetrahydroacridine (LXXXIX) and 15 per cent tetrahydromethylphenanthridine (XC). In the same way, by the use of *m*-hydroxyaniline with acetylcyclohexanone, acetylmethylcyclohexanone, and acetylcamphor, the corresponding hydroxytetrahydroacridines were prepared. It is interesting to note that the anil (XCI) derived from 2-formylcyclohexanone cannot be cyclized. This may be a consequence of improper steric configuration.

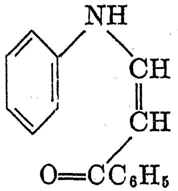


XCI

Several other anils belonging to this general system have failed to cyclize. Claisen and Fischer (50) reported that the anil (XCII) of formylacetophenone gave no quinoline derivatives with concentrated sulfuric acid. Later (51), it

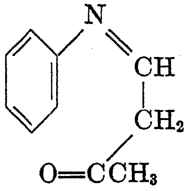


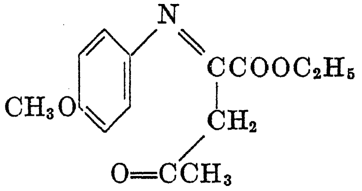
XCII



XCIII

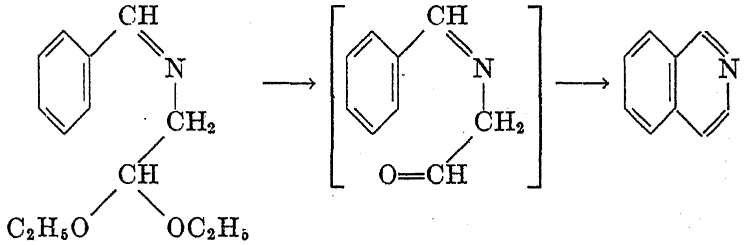
was suggested that the condensation product between formylacetophenone and aniline might have the isomeric structure (XCIII), which could be responsible for the failure of the compound to cyclize. Since all of the anils considered here may have a structure analogous to XCIII, it seems more probable that the explanation is to be found either in incorrect steric configuration or in the presence of a phenyl group attached to the carbonyl. At best, the system could be no better than that of dibenzoylmethane anil (LXXXVI, R = H). The failure (142) of the anil (XCIV) of formylacetone to cyclize seems probably a clear-cut case of wrong steric configuration. It is more difficult to agree with Thielepape (142) that the *p*-aniside of acetone-oxalic ester fails to cyclize for the same reason, since, in this case, any cyclization must take place meta to a methoxyl group, a situation which is known to represent a very unfavorable arrangement.


XCIV


XCV

F. Isoquinoline system

The application of the general reaction to the synthesis of isoquinoline derivatives appears to be limited to the cyclization of simple derivatives of benzaldiminoacetaldehyde (XCVII). The first reported attempt to prepare isoquinoline by this method was made in 1893 by Fritsch (78), who prepared the benzal derivative (XCVI) of aminoacetal, but failed to find the proper conditions for effecting cyclization. The following year, Pomeranz (124) reported that the acetal could be cyclized by sulfuric acid to isoquinoline (XCVIII), presumably through the aldehyde (XCVII). The use of this general synthesis in the preparation of isoquinoline derivatives is shown in table 4. If, instead of a benzaldehyde, a phenyl ketone is condensed with aminoacetal, the product yields a small amount of a 1-substituted isoquinoline (table 4, R_1 = alkyl or aryl) upon cyclization. The most important variations have involved effecting a

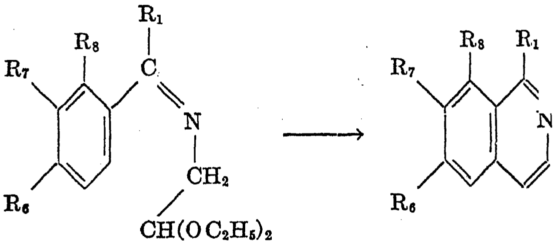

XCVI XCVII XCVIII

similar condensation with substituted benzaldehydes to yield, upon ring closure, isoquinolines with substituents in the benzenoid ring.

The presence of a hydroxyl or alkoxyl group para to the point of expected cyclization seems again a favorable influence, although introduction of a second methoxyl group meta to the point of cyclization produces a compound difficult (R_7 and R_8 = OCH_3) (121) or impossible (R_6 and R_7 = OCH_3) (131) to cyclize. The latter appears odd, in view of the ability of the corresponding methylenedioxy derivative (R_6—R_7 = —O—CH_2—O—) to undergo ring closure. More successful application of the Pomeranz–Fritsch synthesis seems to be limited by the difficulty of finding conditions sufficiently drastic to hydrolyze the acetal but not the aldimine linkage.

TABLE 4

Pomeranz–Fritsch cyclization

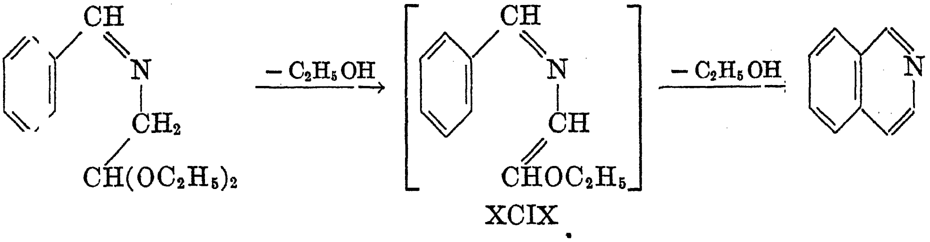


R1	R6	R7	R8	REFERENCES
H	H	H	H	(124, 125)
CH_3	H	H	H	(125)
H	H	H	CH_3	(127)
H	CH_3	H	H	(127)
H	H	H	Cl	(127)
C_6H_5	H	H	H	(127)
H	H	OH*	H	(79, 152a)
H	H	OCH_3	H	(79)
H	H	OC_2H_5	H	(79)
H	—O—CH_2—O—		H	(79)
H	H	OCH_3	OCH_3	(121)
H	H	H	Br	(143)
H	H	Br*	H	(143)
H	Br	H	H	(143)

* Contained some 5-substituted isoquinoline.

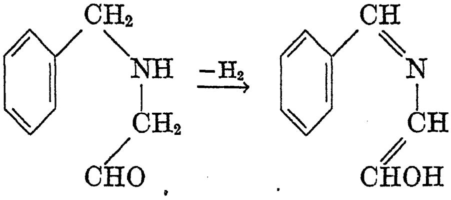
Staub (136) has made a study of several compounds containing the C_6H_5—C—N—C—C grouping and has reached the conclusion that for isoquinoline cyclization, the chain must possess: (*1*) a system of double bonds or the possibility of their formation, (*2*) a hydroxyl or alkoxyl group beta to the nitrogen atom. Staub postulates that the first step in the cyclization of benzaldiminoacetals involves loss of alcohol to give the conjugated system (XCIX). The Fischer (70) cyclization of a benzylaminoacetaldehyde (C) to isoquinoline is

Staub mechanism



XCIX

interpreted as involving preliminary dehydrogenation and formation of the enol (CI).



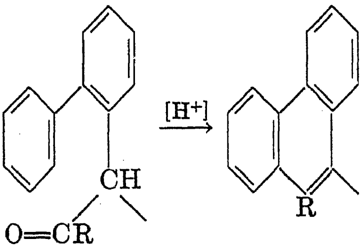
C CI

Without speculation concerning the stage at which dehydrogenation occurs in the Fischer cyclization, it must be conceded that there exists no positive evidence that the Pomeranz–Fritsch synthesis involves direct cyclization of a vinyl ether (XCIX), while every analogy suggests that the intermediate is, as suggested by Pomeranz, benzalaminoacetaldehyde (XCVII), the conjugate acid of which would be expected to cyclize according to the general mechanism.

III. TRICYCLIC SYSTEMS

A. Phenanthrene system

It is possible to conceive of 2-biphenylylacetaldehydes and the corresponding ketones as β-styrylacetaldehydes or methyl ketones in which the double bond

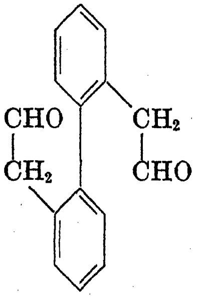


(R = H, alkyl, or aryl)

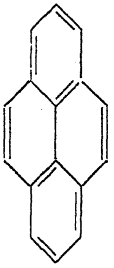
has been incorporated into a benzene ring. Both systems are similar, in that acid-catalyzed cyclodehydration leads to the formation of an aromatic derivative, but there exists considerable difference between the two with respect to yield. Almost uniformly better results are obtained in the phenanthrene as opposed to the naphthalene system, for the elimination of the double bond not only removes a cause for polymerization, but also obviates the possibility of wrong (i.e., *trans*) steric configuration.

Nearly all of the most useful reactions expected to yield 2-biphenylylacetaldehydes or the related ketones involve, as a final step, hydrolysis or rearrangement in the presence of mineral acid. Since these conditions likewise bring about cyclization, in nearly every such experiment, no carbonyl compound is isolated. While this is usually very convenient as applied to synthesis, it does raise questions as to the actual mechanism involved. For the purpose of the present discussion, it will be assumed that a ketone or aldehyde is always involved as an intermediate, and a more complete discussion of the alternative possibilities will be deferred until the subject of mechanism is considered (Part IV).

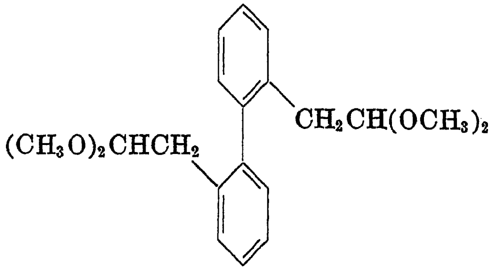
While the preparation of phenanthrene derivatives by this mode of cyclization is a comparatively recent development, the same principle had been applied as early as 1912 by Weitzenbock (151) in an attempt to synthesize pyrene (II). Seeking to prepare the necessary biphenylene-2,2′-diacetaldehyde (I), he hydrolyzed the corresponding acetal (III) with 3 per cent sulfuric acid. Instead of the expected dialdehyde (I) or pyrene (II), he obtained a new aldehyde to which he assigned the structure of 4,5,6,7-dibenzo-Δ-1,4,6-cycloheptatriene-1-aldehyde (IV) rather than that of the partially cyclized product, 4-phenanthrylacetaldehyde (V). This decision was based on the following observations: (*1*) the aldehyde failed to yield pyrene under more energetic treatment with acid; (*2*) it reacted immediately with permanganate solution; (*3*) it reacted readily with bromine to give a stable dibromide (phenanthrene dibromide de-



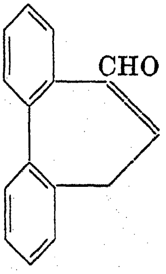
I



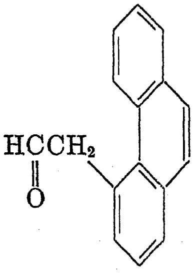
II



III



IV



V

composes on standing); (*4*) oxidation yielded phenanthrenequinone instead of a phenanthrenequinone monocarboxylic acid. The evidence cannot be considered as amounting to a demonstration, and any final decision will have to await an independent synthesis of one of these compounds.

In a series of experiments beginning in 1938, the author and his students have investigated the cyclization of compounds of such a structure that they might be expected to yield derivatives of 2-biphenylylacetaldehyde or 2-biphenylylmethyl ketones (VI, R = H, alkyl, or aryl) under conditions of the cyclization.

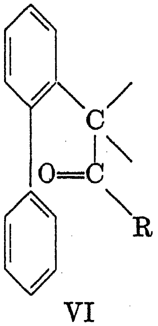
TABLE 5

Cyclization of mono ethers of 2-biphenylylethylene glycol (VII)

A. Methyl ethers

R	R′	YIELD*	REFERENCES
		per cent	
H	H	46	(44)
CH_3	H	50	(43)
C_2H_5	H	53	(30)
n-C_3H_7	H	51	(30)
n-C_4H_9	H	40	(30)
$C_6H_5CH_2$	H	70	(30)
C_6H_5	H	†	(38)
p-$CH_3C_6H_4$	H	†	(38)
B. Phenyl ethers			
CH_3	H	32*	(43)
C_6H_5	H	84	(38)
C_6H_5	CH_3	72*	(37)
C_6H_5	C_2H_5	70	(37)
C_6H_5	C_6H_5	62*	(37)

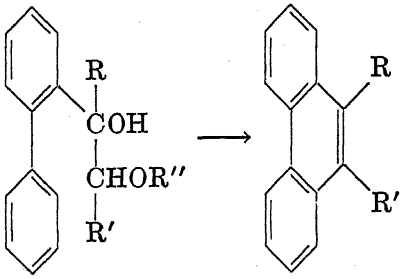
* Over-all; carbinol not isolated.
† Not reported.



VI

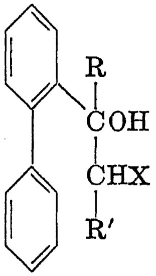
(R = H, alkyl, or aryl)

One such type embraces the monomethyl or phenyl ethers of 2-biphenylylethylene glycol (VII, R″ = CH_3 or C_6H_5). The results are summarized in table 5.



VII

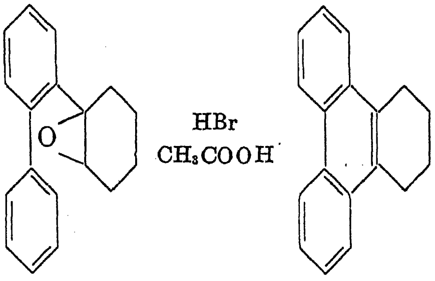
Several other experiments, mostly of an exploratory nature, were tried with the similar systems (VIII) in which the β-carbon atom of the chain contained some group other than methoxyl or phenoxyl. These include the hydroxyl (45), β-naphthoxyl (43), and diethylamino (43) groups, all of which undergo cyclization to some extent.



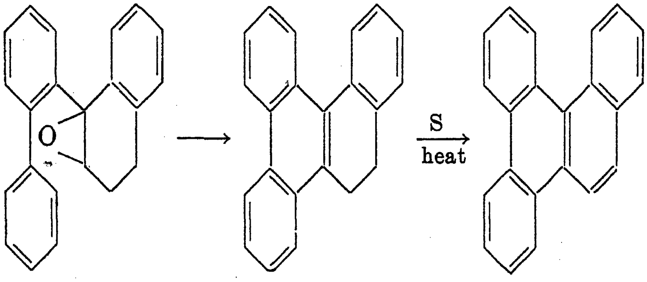
VIII

(X = OH, β-$OC_{10}H_7$, $N(C_2H_5)_2$)

More important than any of the methods noted is the cyclization of olefin oxides. The first example (26) was the cyclization of the oxide (IX) derived from (2-biphenylyl)cyclohexene-1 to yield 9,10-cyclohexenophenanthrene. This general method has been applied with the oxides of several cycloölefins. The cyclization of 1-(2-biphenylyl)-3,4-dihydronaphthalene oxide (XI), for example, affords the most direct route to the carcinogenic 1,2,3,4-dibenzophenanthrene (XII) (34). Other examples will be found in table 6.



IX X



XI XII

The olefin oxide cyclization is equally applicable to the preparation of alkyl, dialkyl, and alkyl-aryl phenanthrenes, using non-cyclic 2-biphenylyl olefins.

TABLE 6

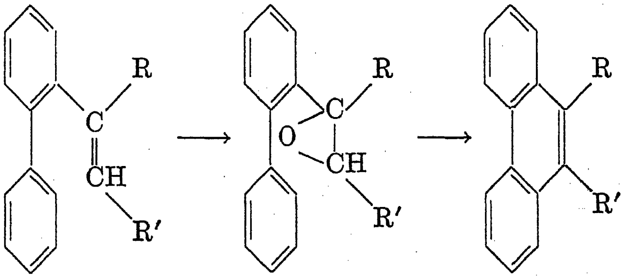
OXIDE OF 1-(2-BIPHENYLYL)-	CYCLIZATION PRODUCT	YIELD*	REFERENCES
		per cent	
Cyclopentene	9,10-Cyclopentenophenanthrene	16	(26)
Cyclohexene	9,10-Cyclohexenophenanthrene	30, 100	(26, 36)
3,4-Dihydronaphthalene	1,2,3,4-Dibenzo-9,10-dihydronaphthalene	51†	(34)
4-Methyl-3,4-dihydronaphthalene	9-Methyl-9,10-dihydro-1,2,3,4-dibenzophenanthrene	89.5	(35)
3-Methyl-3,4-dihydronaphthalene	10-Methyl-9,10-dihydro-1,2,3,4-dibenzophenanthrene	73	(35)
3,4-Dimethyl-3,4-dihydronaphthalene	9,10-Dimethyl-9,10-dihydro-1,2,3,4-dibenzophenanthrene	‡	(35)

* Unless otherwise noted, all yields are over-all for both oxidation and cyclization.

† Yield of 1,2,3,4-dibenzophenanthrene after dehydrogenation.

‡ Not reported.

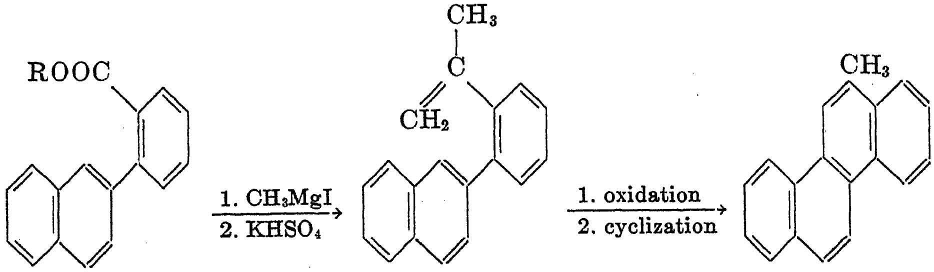
The over-all yield of phenanthrene hydrocarbon (XIV) obtained from the olefin (XIII) varies from 2 per cent in the case of phenanthrene (R and R′ = H) to 69 per cent in the case of 9-isopropylphenanthrene (R = H; R′ = i-C_3H_7). The



XIII XIV

results are summarized in table 7.

This type of cyclization has been applied to the synthesis of 6-methylchrysene (33). When 2′-carbomethoxy-2-phenylnaphthalene (XV) was treated with methylmagnesium iodide and the resulting carbinol dehydrated, the olefin (XVI) which resulted was readily oxidized and cyclized to 6-methylchrysene (XVII).



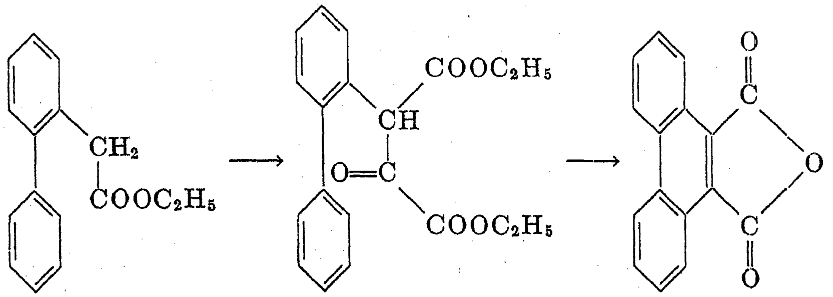
XV XVI XVII

TABLE 7

Cyclization of simple olefin oxides

R	R′	YIELD FROM OLEFIN	REFERENCES
		per cent	
H	H	2	(44)
H	C_2H_5	43	(30)
H	n-C_3H_7	66	(30)
H	i-C_3H_7	69	(30)
H	n-C_4H_9	34	(30)
H	n-C_5H_{11}	53	(30)
CH_3	H	40	(31)
C_2H_5	CH_3	54	(31)
n-C_3H_7	C_2H_5	44	(31)
n-C_4H_9	n-C_3H_7	67	(31)
n-C_5H_{11}	H	31	(32)
CH_3	CH_3	39	(32)
C_6H_5	n-C_3H_7	64	(32)
C_6H_5	n-$C_{10}H_{21}$	39	(32)

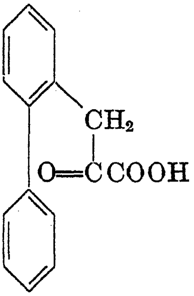
The first report of the successful cyclization of a 2-biphenylylmethyl ketone derivative was that of Schönberg and Warren (133), who found that ethyl α-keto-β-(2-biphenylyl)succinate (XIX), obtained by the condensation of



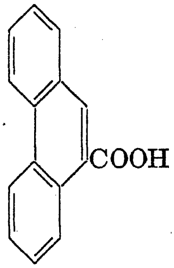
XVIII XIX XX

ethyl oxalate with ethyl biphenylyl-2-acetate (XIX) in the presence of potassium, underwent cyclization in 95 per cent sulfuric acid to give a good yield of phenanthrene-9,10-dicarboxylic anhydride (XX). This is an unusual example of the Bougault reaction (5, 25) in that, instead of a hydroaromatic derivative, a fully aromatic compound is formed directly.

In an independent investigation, Geissmann and Tess (80) observed that the keto ester (XIX) could be cyclized in a boiling mixture of hydrobromic and acetic acids. Under these conditions, a mixture consisting chiefly of phenanthrene-9-carboxylic acid (XXII) together with a small amount of the 9,10-dicarboxylic acid was obtained. The formation of the monocarboxylic acid (XXII) seems best explained by assuming the formation of an intermediate keto acid (XXI) by hydrolysis and decarboxylation of the oxalyl ester (XIX). A comparable transformation has been observed by Fieser and Holmes in a related system (67, 68).

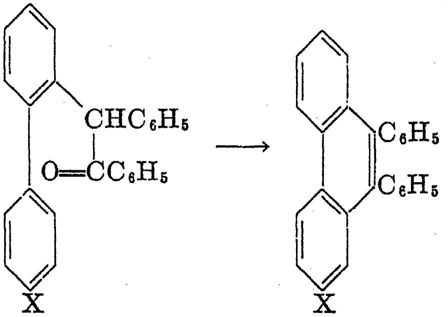


XXI



XXII

Finally, the only simple ketones which have been cyclized to phenanthrene derivatives are α-(2-biphenylyl)desoxybenzoin (XXIII, X = H) and its 4′-chloro derivative, which are readily cyclized by either sulfuric acid or the usual hydrobromic–acetic acid mixture to yield the corresponding 9,10-diphenylphenanthrenes (XXIV) (46).

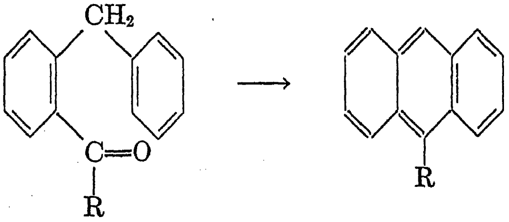


XXIII XXIV

(X = H or Cl)

B. *Anthracene system*

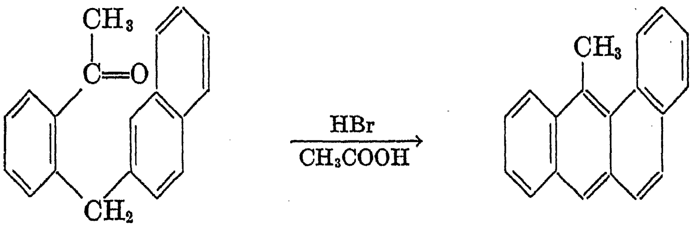
The most recently discovered of all aromatic cyclodehydrations is that yielding anthracene derivatives from *o*-benzylphenyl aldehydes and ketones (XXV, R = H, alkyl, or aryl). As might be predicted from the known stability of aromatic aldehydes and ketones, side reactions are, in general, less important than those encountered in the naphthalene and phenanthrene systems.



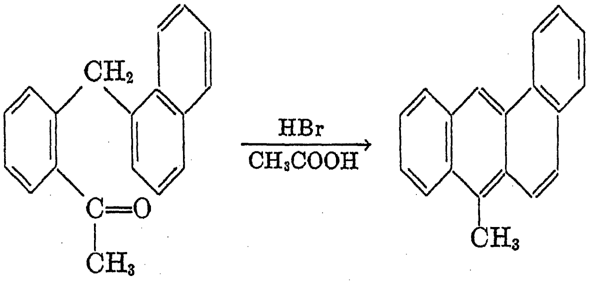
XXV XXVI

The first example of this type of cyclization was observed by Bergmann (11), who found that hydrolysis of the acetal of *o*-benzylbenzaldehyde (XXV, R = H) gave not only the expected aldehyde, but also a small quantity of anthracene.

It was felt (27) that this could not be a modification of the Elbs synthesis, as claimed by Bergmann, but rather an acid-catalyzed cyclodehydration such as those which were already known in the naphthalene and phenanthrene systems. The reaction was extended to *o*-benzylphenyl ketones (XXV, R = alkyl or aryl), resulting in the formation of meso alkyl- and aryl-anthracenes (27). It was pointed out that this reaction might form a useful tool in the introduction of meso alkyl groups into systems containing the anthracene nucleus. This was illustrated by the synthesis of the carcinogenic 9-methyl- and 10-methyl-1,2-benzanthracenes (XXVII and XXVIII) (28).



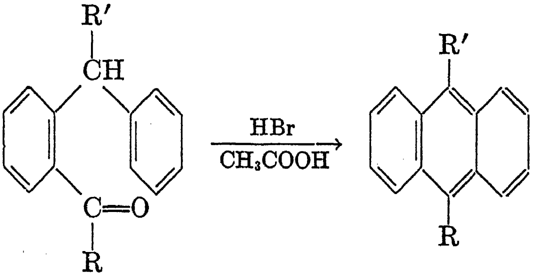
XXVII



XXVIII

Another extension of the synthesis can be effected by introduction of a substituent in the alpha position of the benzyl group of the *o*-benzylphenyl ketone

(XXIX), yielding an anthracene derivative with substituents in both meso-positions (XXX).



XXIX XXX

TABLE 8

Cyclization of o-benzylphenones (XXIX → XXX)

R	R′	TIME	YIELD	REFERENCES
		hours	*per cent*	
H	H	6	0.4*	(11)
CH_3	H	96	80	(27)
C_2H_5	H	96	69	(27)
C_6H_5	H	96	75	(27)
CH_3	C_6H_5	264	50†	(40)
C_2H_5	C_6H_5	264	47.5†	(40)
C_6H_5	C_6H_5	240	81	(40)
CH_3	CH_3			(13)
C_2H_5	CH_3			(13)
n-C_3H_7	CH_3			(13)
n-C_4H_9	CH_3			(13)
n-C_5H_{11}	CH_3			(13)
n-C_6H_{13}	CH_3			(13)
C_6H_5	CH_3			(13)
$C_6H_5CH_2$	CH_3			(13)

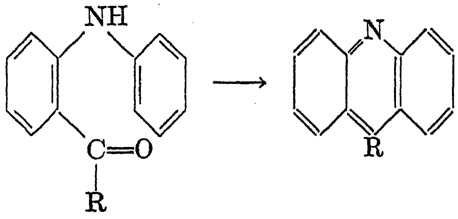
* Calculated from acetal. No effort made to carry reaction to completion.
† Over-all yield from nitrile.

9-Phenyl-10-alkyl- and aryl-anthracenes (XXX, R′ = C_6H_5, R = alkyl or aryl) were prepared in this way (40). More recently, Berliner (13) has shown that the reaction can be extended to *o*-(α-phenylethyl)phenyl ketones (XXIX, R′ = CH_3) to yield 9-methyl-10-alkyl- and aryl-anthracenes (XXX, R′ = CH_3, R = alkyl or aryl).

The results of the anthracene cyclization are summarized in table 8.

C. Acridine system

The nitrogen analog of the anthracene synthesis is the cyclization of *o*-anilinobenzaldehydes or *o*-anilinophenyl ketones (XXXI, R = H, alkyl, or aryl) to yield acridine derivatives (XXXII). The older of the two, the acridine synthesis,

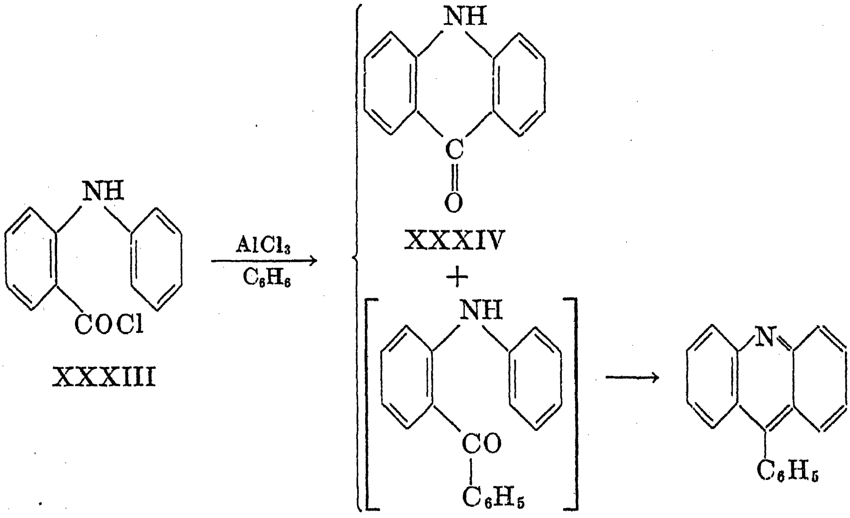


XXXI XXXII

(R = H, alkyl, or aryl)

has proved a versatile tool, and many complex and highly substituted derivatives have been prepared by its use.

The first instances of this reaction, encountered in the anthraquinone series (9, 59), were complex in character and the general nature of the reaction was not appreciated until a few years later (1906), when Ullmann and Ernst (145) came upon an accidental instance of this cyclization. When the acid chloride (XXXIII) of *N*-phenylanthranilic acid in benzene solution was treated with aluminum chloride, not only acridone but also 9-phenylacridine (XXXVI) was

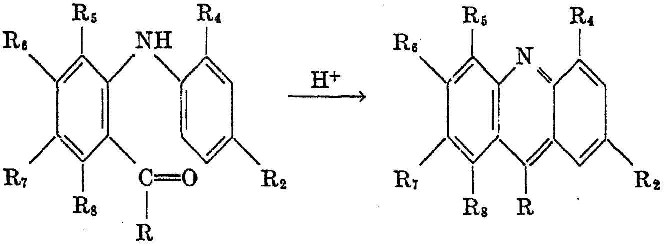


XXXIII XXXIV XXXV XXXVI

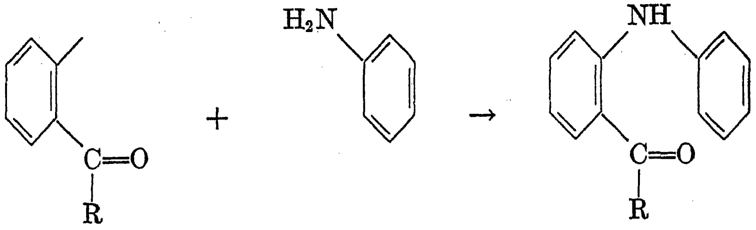
obtained. Ullmann and Ernst concluded that the intermediate in the formation of the latter compound was 2-phenylaminobenzophenone (XXXV), and they were able to show that such a cyclization could be effected by the use of sulfuric acid in acetic acid solution. Ullmann and his coworkers prepared many substituted 2-phenylaminobenzophenones containing substituents in either or both nuclei and showed that these ketones could be cyclized. Mayer and coworkers (107, 108, 109), a few years later, showed that the same reaction could be extended to *o*-phenylaminobenzaldehydes. A summary of their results, as well as those of other workers in the field, will be found in table 9.

The general methods by which the required phenylamino phenones and benzaldehydes were obtained involved either the condensation of an arylamine (XXXVIII) with an *o*-halophenone or *o*-halobenzaldehyde (XXXVII, R = H, alkyl, or aryl) or of a halogen derivative (XL) with an *o*-aminophenone or *o*-aminobenzaldehyde (XXXIX).

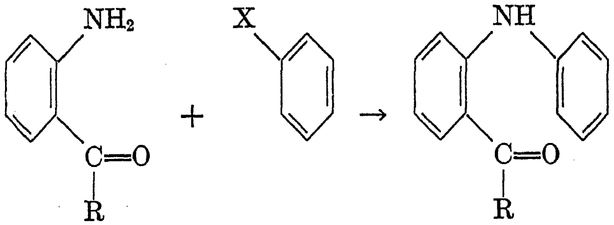
TABLE 9



R	R_2	R_4	R_5	R_6	R_7	R_8	REFERENCES
H	CH_3	H	H	H	H	H	(96)
H	H	CH_3	H	H	H	H	(96)
H	OC_2H_5	H	H	H	H	H	(97)
H	H	OCH_3	H	H	H	H	(97)
H	NO_2	H	H	H	H	H	(96)
H	H	NO_2	H	H	H	H	(96, 109)
H	CH_3	NO_2	H	H	H	H	(109)
H	Cl	NO_2	H	H	H	H	(108)
H	NO_2	NO_2	H	H	H	H	(109)
CH_3	CH_3	H	H	H	H	H	(97)
CH_3	NO_2	H	H	H	H	H	(97)
CH_3	H	H	H	H	NO_2	H	(97)
C_6H_5	H	H	H	H	H	H	(107)
C_6H_5	CH_3	H	H	H	H	H	(97)
C_6H_5	OC_2H_5	H	H	H	H	H	(97)
C_6H_5	NO_2	H	H	H	H	H	(97)
C_6H_5	H	NO_2	H	H	H	H	(97)
C_6H_5	H	H	H	NO_2	H	H	(145)
C_6H_5	CH_3	NO_2	H	H	H	H	(107)
C_6H_5	Cl	NO_2	H	H	H	H	(107)
C_6H_5	NO_2	NO_2	H	H	H	H	(107)
C_6H_5	NH_2	H	H	NO_2	H	H	(145)
C_6H_5	H	H	H	NO_2	H	NO_2	(144)
C_6H_5	CH_3	NO_2	H	CH_3	H	H	(107)
C_6H_5	NO_2	NO_2	H	CH_3	H	H	(107)
C_6H_5	NO_2	NO_2	H	H	CH_3	H	(107)
C_6H_5	OCH_3	NO_2	NO_2	H	H	H	(130)
C_6H_5	NH_2	H	H	NO_2	H	NO_2	(144)
C_6H_5	H	OH	H	NO_2	H	NO_2	(144)
p-$CH_3OC_6H_4$	H	H	H	NO_2	H	H	(145)



XXXVII XXXVIII

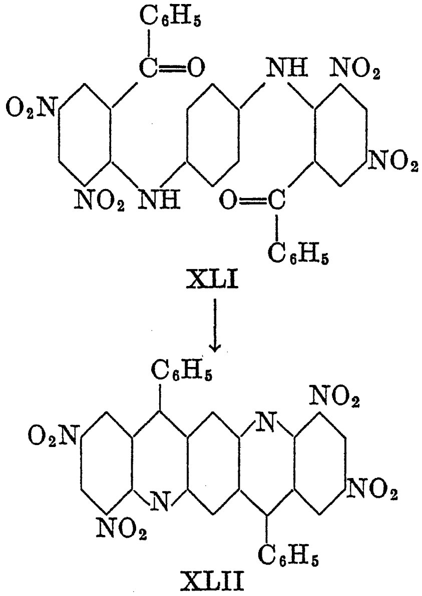


XXXIX XL

Not infrequently, nitro groups are introduced in positions ortho and/or para to the halogen atoms (XXXVII and XL), greatly increasing the ease of condensation. These condensations are usually brought about by heating the reactants at a reasonably high temperature (150–220°C.), usually in the presence of sodium or potassium carbonate. Under these conditions, it is not uncommon for a small quantity of the arylamino ketone or aldehyde formed to cyclize to the acridine. In the majority of the cases reported, the arylamino ketone or aldehyde was not purified, but used directly in the cyclization. In the absence of the carbonate, more cyclization takes place directly, but the yield of cyclized material is usually not high. If it is desired to bring about condensation and cyclization in a single operation, good yields may be obtained by using sodium acetate. Several instances of this direct cyclization have been recorded, though they are not so noted in the tables, the assumption being made that the ketone is the intermediate in each case. In the great majority of the cases, cyclization was effected as a separate step using sulfuric acid, either concentrated or diluted with acetic acid.

From table 9, it may be seen that ring closure is not prevented by the presence of alkoxyl groups meta to the point of expected cyclization and that acridine derivatives containing a variety of substituents may be prepared by this process. The 1,2- and 3,4-benzacridines prepared by this general method have been listed separately in tables 10 and 11.

In addition to the foregoing, there are several instances of cyclization of this general type which do not lend themselves well to tabular presentation. Ullmann and Broido (144) found that *p*-phenylenediamine could be condensed with 2 moles of 2-chloro-3,5-dinitrobenzophenone to give XLI, which underwent cyclization to yield the pentacyclic derivative (XLII).



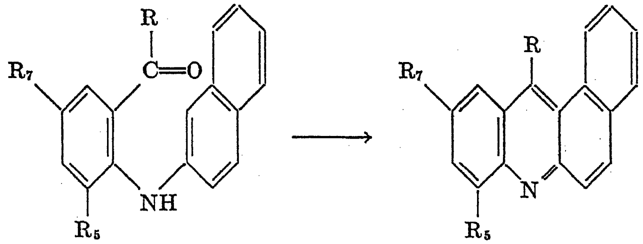
XLI

XLII

Stimulated by a desire to prepare substances capable of being used as, or converted to, dyes, investigators have prepared many acridine derivatives from the anthraquinone nucleus by carbonyl cyclization. These can be divided into two groups: those which involve one or both of the carbonyls of the anthraquinone, and those which do not.

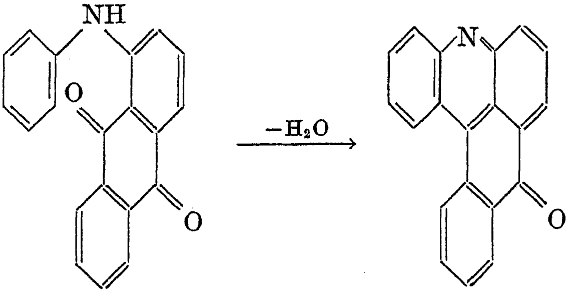
TABLE 10

1,2-Benzacridines



R	R_5	R_7	REFERENCES
CH_3	H	H	(12)
C_6H_5	H	H	(107)
C_6H_5	H	NO_2	(145)
C_6H_5	NO_2	NO_2	(144)

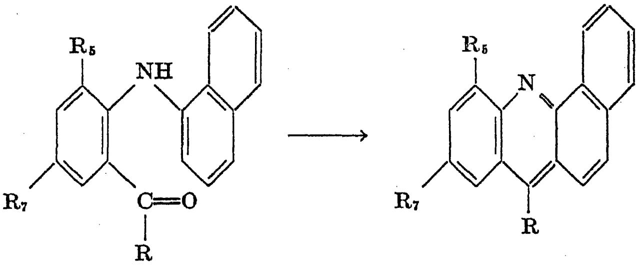
The first recorded instance of the formation of an acridine nucleus by aromatic cyclodehydration belongs to the former type. Damman and Gattermann (59) showed that 1-phenylaminoanthraquinone (XLIII) could be cyclized to yield a compound (XLIV) containing an acridine nucleus. This type of synthesis has been further extended (9, 76).



XLIII XLIV

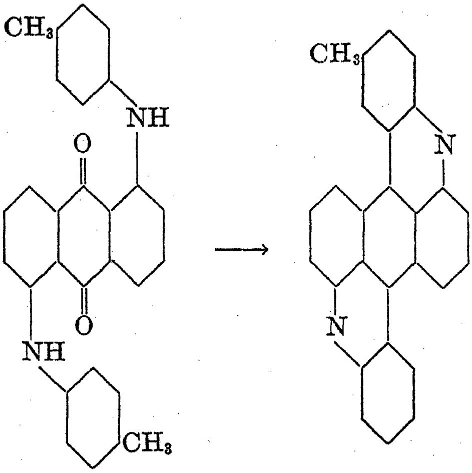
TABLE 11

3,4-Benzacridines



R	R_5	R_7	REFERENCES
CH_3	H	H	(12)
C_6H_5	H	H	(107)
C_6H_5	H	NO_2	(145)
C_6H_5	NO_2	NO_2	(144)

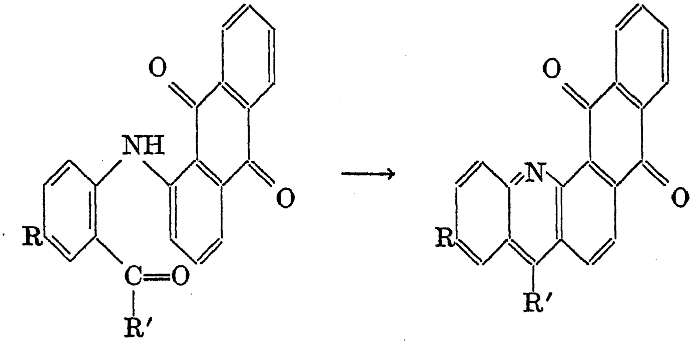
The patent literature reveals claims that both carbonyls of an anthraquinone nucleus can be used in such a cyclization. Thus, 1,5-di(*p*-toluidino)-anthraquinone (XLV) (9) may be cyclized to a compound (XLVI) which



XLV XLVI

contains two acridine nuclei. This is claimed to be general for 1,5-diarylaminoanthraquinones.

The other major class of "acridinated" anthraquinones consists of those in which the carbonyl groups of the quinone do not take part in the cyclization, condensation being effected instead with the terminal nuclei. Mayer and Freund (107) observed that derivatives of 1-(*o*-acylbenzamino)anthraquinones (XLVII) would undergo cyclization to yield acridine derivatives (XLVIII). Even more complex examples (10, 107) based on the same principles have been cited.

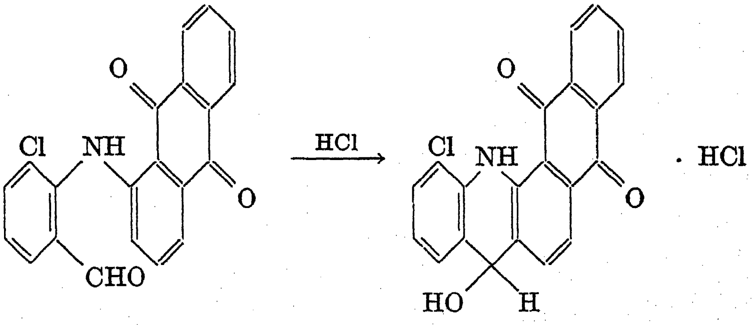


XLVII XLVIII

($R = H$; $R' = C_6H_5$)

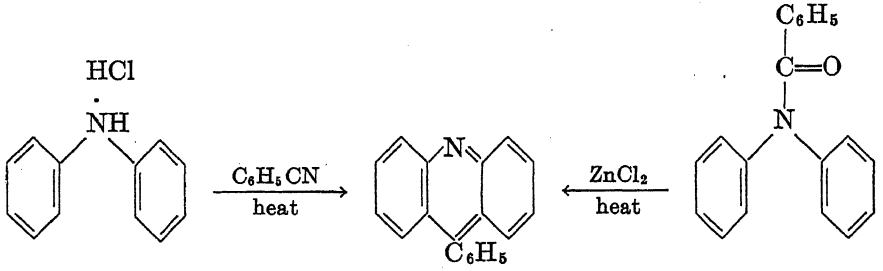
($R = CH_3$; $R' = CH_3$)

When 1-(*o*-formylbenzamino)anthraquinones (XLIX) are used, the rea involved does not follow the general pattern, but appears, instead, to be ar at what might be called an "intermediate" stage to yield 9,10-dihydroac derivatives (L) (108, 109). It is probable that all of the acridine cycliza which have been discussed pass through such a stage, elimination of water the final step. Berliner (12) has presented an electronic mechanism fo acridine cyclization.



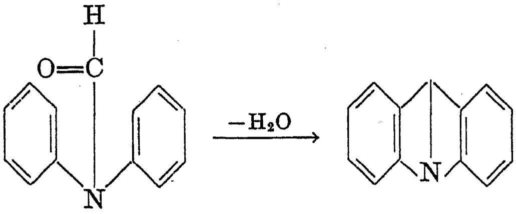
XLIX L

In addition to the acridine synthesis already discussed, there are several others which may involve aromatic cyclodehydration. The first of these is the Bernthsen synthesis. Bernthsen found that heating diphenylamine hydrochloride with benzonitrile (14) or heating *N*-benzoyldiphenylamine (LII) with zinc chloride (15) yielded a base which was shown (17) to be 9-phenylacridine (LI). Similar results (17) were obtained by heating diphenylamine with benzoic acid and zinc chloride. In the same way, acetic (16, 17, 19, 73) or formic (17) acid with diphenylamine and zinc chloride gave 9-methylacridine and acridine, respectively. The mechanism proposed by Bernthsen and Bender (17) postulates the formation of a para bond.

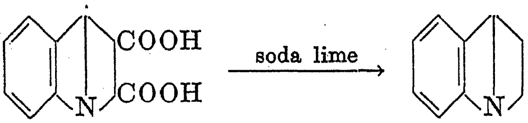


LI LII

Bernthsen and Bender mechanism

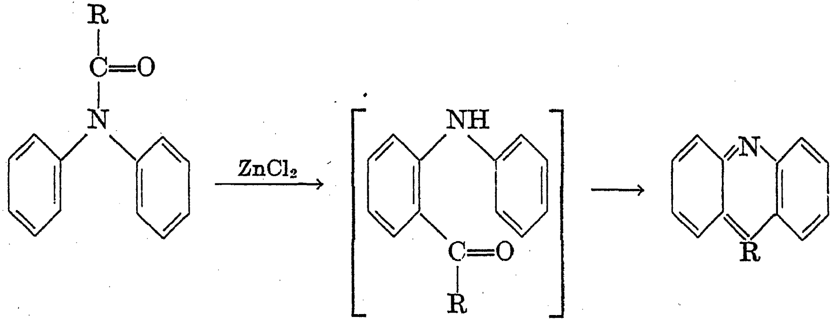


Since oxidation of acridine yields a dicarboxylic acid (LII) which may be decarboxylated to yield quinoline, they were convinced that quinoline likewise had a para bond.

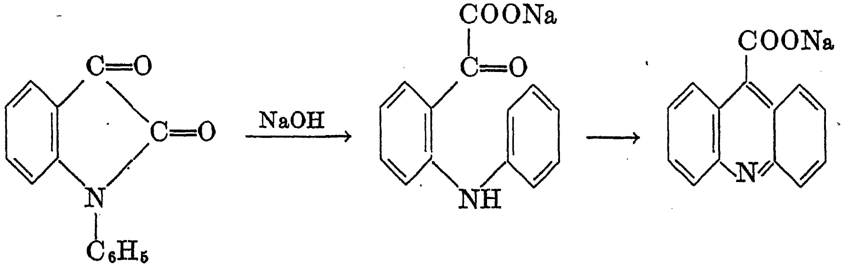


LIII

In view of the evidence (4) that no such bond exists in acridine, a more likely mechanism involves the preliminary migration of the acyl group to the ortho-position of one of the benzene nuclei, followed by aromatic cyclodehydration.



The last type of cyclization which will be considered is that involved when *N*-phenylisatin (LIV) is refluxed with a sodium hydroxide solution (75). The keto acid salt (LV) first formed cyclizes to yield the salt of acridine-9-carboxylic acid (LVI). Stollé (141) has applied this synthesis to the preparation of benz- and dibenz-acridinecarboxylic acids. If this reaction proceeds by the same mechanism as the acid-catalyzed cyclization previously considered, it may be a consequence of an activating influence of the carboxylate ion which makes possible cyclization at a lower hydrogen-ion concentration than is required in the previous cases.



LIV LV LVI

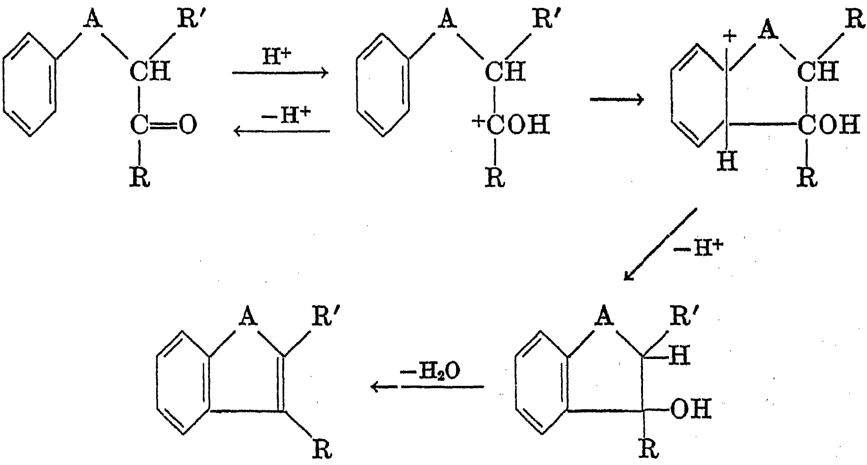
IV. MECHANISM

A. General mechanism

In discussing the mechanism for aromatic cyclodehydration, the general mechanism will be considered first and then two special cases—the phenanthrene and the anthracene cyclizations—considerable justification for their particular mention being found in the amount of pertinent evidence now available.

Turning to the general reaction, the first step probably involves the addition of

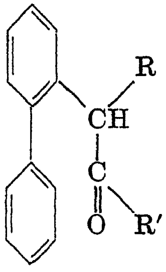
Mechanism for aromatic cyclodehydration



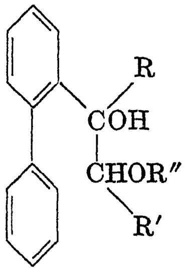
a proton to the carbonyl oxygen, followed by an electrophilic attack upon the benzenoid ring by the positively charged carbon. Following restoration of benzenoid character to the ring by loss of a proton, the carbinol is dehydrated with the creation of a new double bond.

B. Phenanthrene system

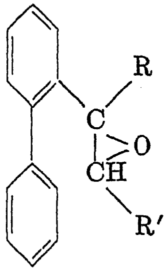
The cyclization of 2-biphenylylmethyl ketones (I) raises no problem which cannot be dealt with in terms of the general mechanism. This ability to yield phenanthrene derivatives is shared by two other types of compounds,—namely, 2-biphenylylglycols and their ethers (II) and olefin oxides (III). In view of the known ability of glycols and olefin oxides to rearrange to aldehydes and ketones, the mechanism for this transformation might seem too obvious to warrant further discussion, though a detailed analysis of the possibilities will show that these types of cyclization may be a good deal more complex in character. While such an analysis may be made for each of these, attention will be centered on the more important biphenylylolefin oxides (III).



I



II

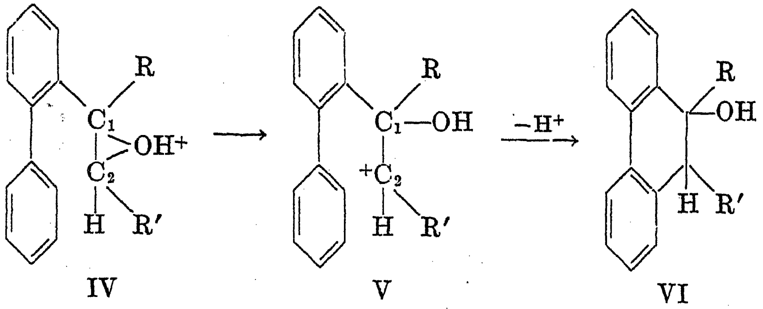


III

(R″ = H, alkyl, or aryl)

The first step in cyclization is undoubtedly the addition of a proton to the oxygen to yield the conjugate acid (IV). The second step is a scission of the bond between either the C_1 (first) or C_2 (second) carbon atom of the chain and the

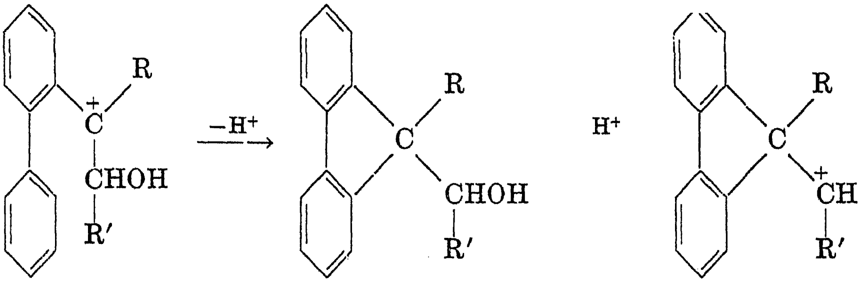
"Direct" mechanism



oxide oxygen. In the simpler and more improbable case, we can imagine that the C_2-to-oxygen bond has been broken, leaving C_2 with a positive charge (V). This could attack the adjacent benzenoid ring, yielding a 9-hydroxy-9,10-dihydrophenanthrene derivative which could simply lose the elements of water to become fully aromatic. While this "direct" mechanism is appealing in its simplicity, it is probably erroneous since oxides, like glycols, tend to dissolve that carbon-to-oxygen bond which bears the most potent electron-releasing groups (93a), in this case, C_1.

If the hypothesis that it is the C_1-oxygen bond that is broken is accepted, there still remain several plausible routes to the phenanthrene hydrocarbon. The carbonium ion (VII) may stabilize itself in any one of at least three ways. It may attack the adjacent aromatic ring electrophilically, yielding a "fluorene carbinol" (VIII). Present evidence (48, 110, 152) seems to indicate that fluorene carbinols of type VIII (R = R′), upon dehydration, rearrange to yield phenanthrene derivatives, evidently through the intermediate IX.

"Fluorene carbinol" mechanism

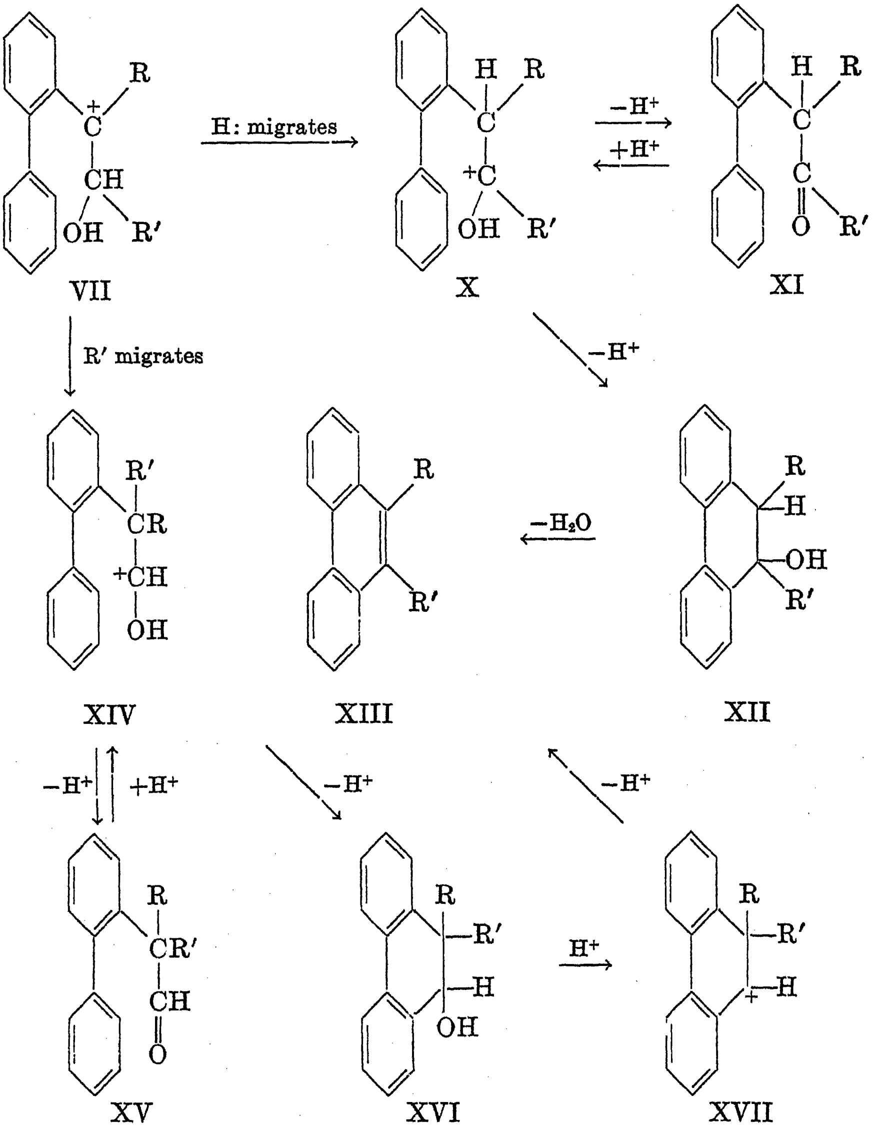


VII VIII IX

Another way in which the carbonium ion (VII) may achieve stability is by rearrangement, the hydrogen or R′ group migrating to C_1. If we assume the migration of the hydrogen atom with an electron pair, the result is the conjugate acid (X) of the ketone (XI). The cyclization of this conjugate acid (X) yields a dihydrophenanthrene derivative (XII), which is immediately dehydrated to the fully aromatic compound (XIII).

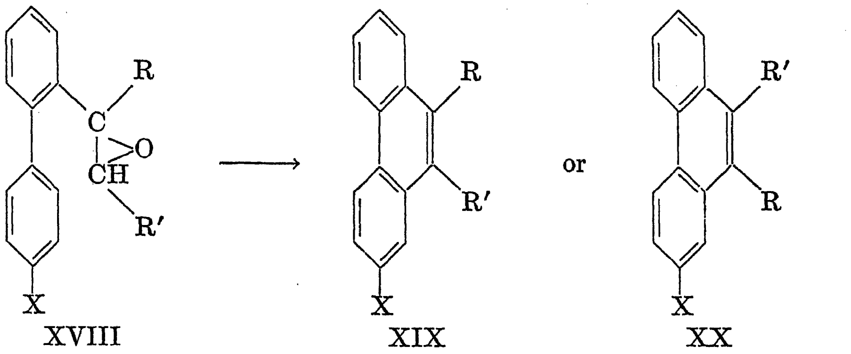
If, instead of hydrogen, the R′ group of the original carbonium ion (VII) migrates with a pair of electrons, the product is the conjugate acid (XIV) of the aldehyde (XV). Cyclization of the conjugate acid (XIV) yields the 9,10-dihydrophenanthrol (XVI), which may be dehydrated, with rearrangement, to yield the phenanthrene hydrocarbon (XIII), the ion (XVII) probably being formed as an intermediate. If R and R′ are different, the group which migrates in the first rearrangement (VII → XIV) may not be that which migrates in the second (XVII → XIII). A forseeable consequence of such a double rearrangement is that in the cyclization of a 2-biphenylylolefin oxide (XVIII) in which the biphenylyl group is unsymmetrically substituted, either or both of two possible phenanthrene cyclization products (XIX or XX) might be obtained.

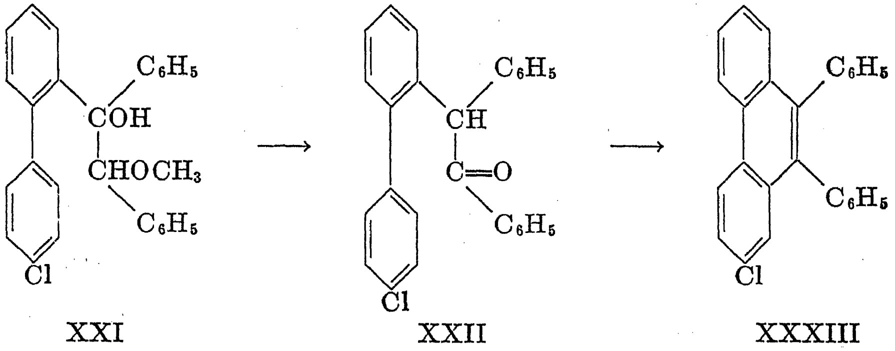
"Carbonyl" mechanism

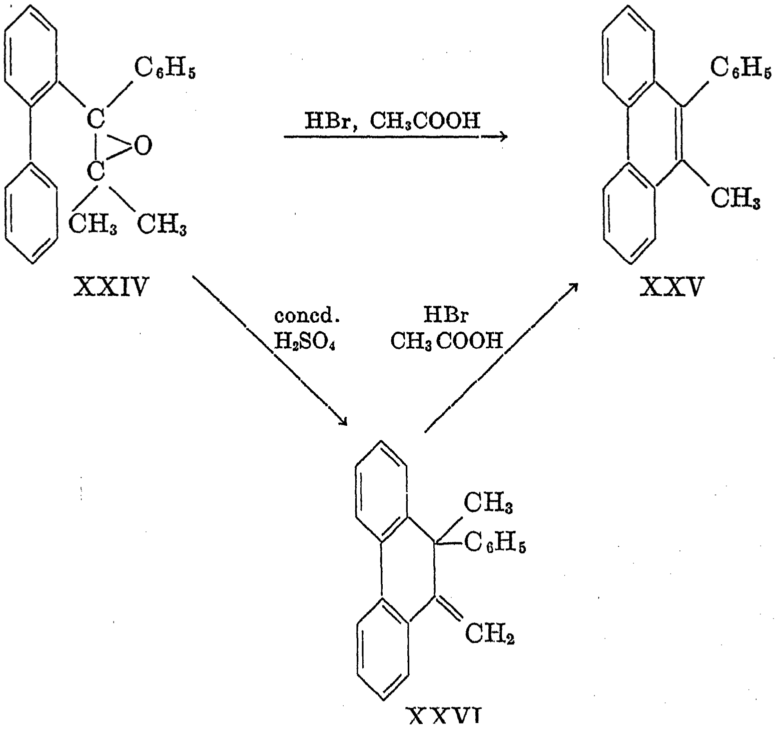


What evidence exists, points to the "carbonyl" mechanism as opposed to the "direct" or "fluorene carbinol" mechanisms. It has been found (46) that the cyclization of 1,2-diphenyl-1-(4-chlorobiphenyl-2-yl)-2-methoxyethanol-1 (XXI) to 2-chloro-9,10-diphenylphenanthrene (XXIII) involves as an intermediate the ketone XXII.

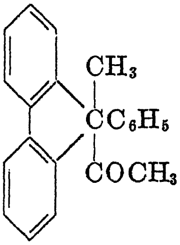
Another observation (36) made in connection with the olefin oxide type of cyclization involves the oxide (XXIV) of 1-phenyl-1-(2-biphenylyl)-2-methylpropene-1, which loses a methyl group when refluxed with hydrobromic and acetic acids, yielding 9-phenyl-10-methylphenanthrene (XXV). With cold


R
C
O
CH
R′
X
XVIII
R
R′
X
XIX
or
R′
R
X
XX


C6H5
COH
CHOCH3
C6H5
Cl
XXI
C6H5
CH
C=O
C6H5
Cl
XXII
C6H5
C6H5
Cl
XXXIII


C6H5
C
O
C
CH3 CH3
XXIV
HBr, CH3COOH
C6H5
CH3
XXV
concd.
H2SO4
HBr
CH3COOH
CH3
C6H5
CH2
XXVI

concentrated sulfuric acid, the product is believed to be 9-methyl-9-phenyl-10-methylene-9,10-dihydrophenanthrene (XXVI). This hydrocarbon likewise yields 9-phenyl-10-methylphenanthrene (XXV) when refluxed with boiling hydrobromic and acetic acids. A possible explanation for the formation of XXVI is that in concentrated sulfuric acid, at least, the first reaction is the rearrangement of the oxide to a ketone (XXVII), followed by cyclization and elimination of the elements of water.

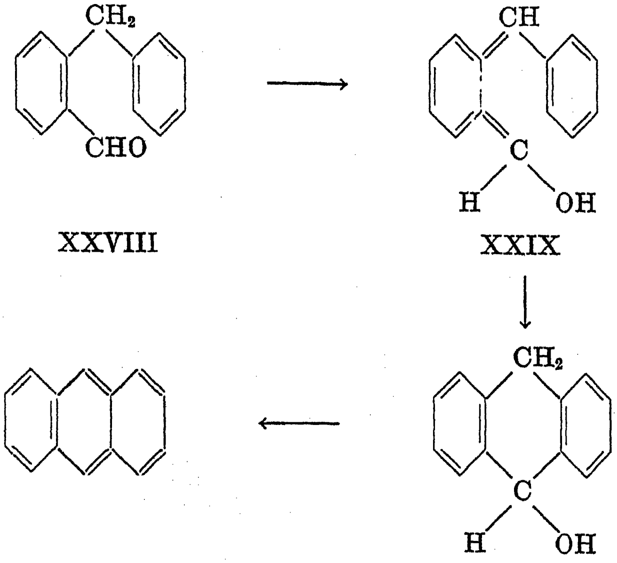


XXVII

C. Anthracene system

In addition to characterizing the cyclization of *o*-benzylbenzaldehyde (XXVIII) as "a modification of the Elbs synthesis of anthracene derivatives," Bergmann (11) proposed a mechanism based on that suggested by Cook (56) for the Elbs reaction.

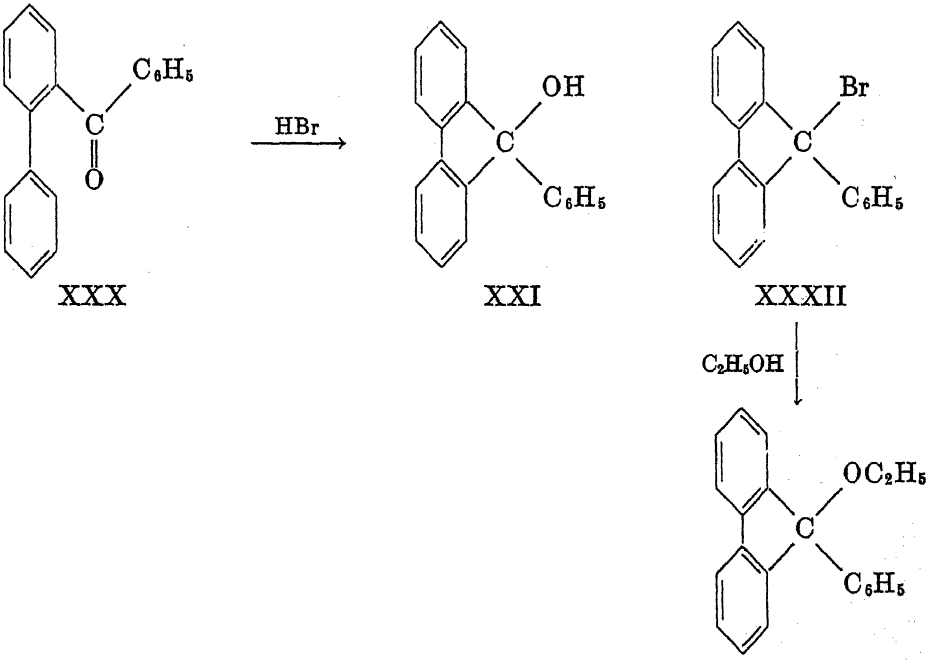
Bergmann mechanism



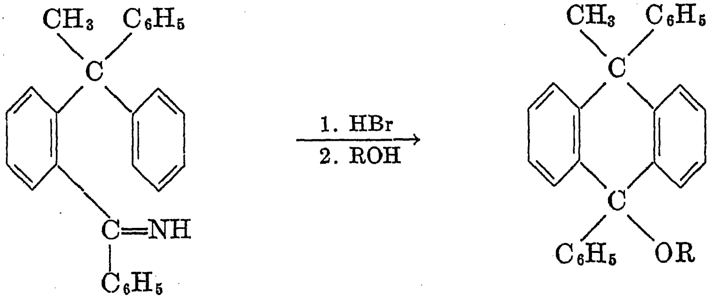
The Bergmann mechanism, later erroneously attributed (12) to Bradsher, involves enolization, cyclization with transannular migration of hydrogen, and finally dehydration. The author's opinion (27) that this acid-catalyzed cyclization cannot properly be considered an example of the Elbs pyrolysis has received the support of Fieser (65), although apparently not that of Cook (57).

The author suggested as a "satisfactory working hypothesis" that the intermediate in the cyclization was the enol (XXIX), which cyclized directly by loss of water in direct analogy to the mechanism proposed by Carter and Van Loon (55) for the dimolecular cyclization of β-phenylacetaldehyde (see Section II, A).

In conjunction with Smith (39, 41), an investigation was carried out which demonstrated by cyclization of *o*-phenylbenzophenone (XXX) to a fluorene derivative that enolization was not necessary for cyclization. In order to prevent the further condensation of the fluorenol believed to be first formed, the reaction was carried out in 48 per cent hydrobromic acid to effect conversion of the carbinol to the more stable bromide (XXXII). The latter was identified by conversion to 9-ethoxy-9-phenylfluorene (XXXIII) by the action of ethyl alcohol.



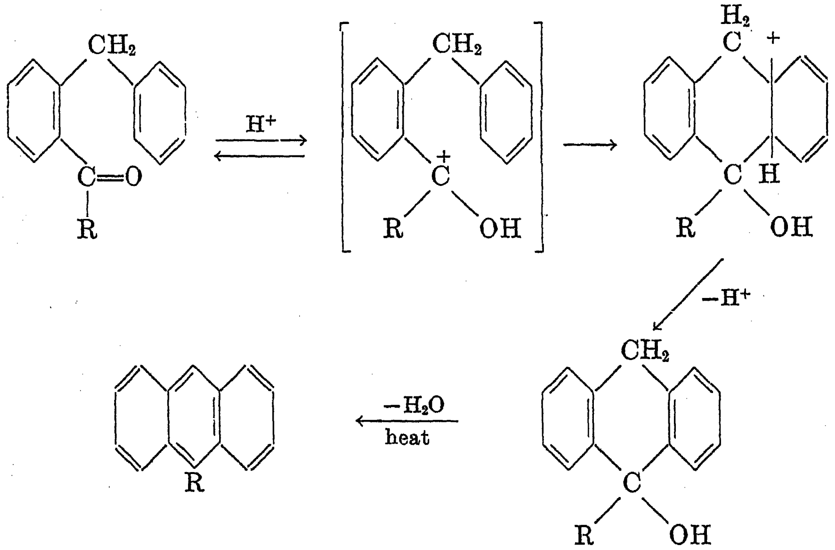
Since it might be objected that the formation of a five-membered ring is not exactly comparable to the formation of a six-membered ring, the imine (XXXIV) was prepared and subjected directly to the action of hydrobromic acid. The crude bromide obtained was refluxed with methyl and ethyl alcohols to yield compounds having the composition of the expected ethers (XXXV, R = CH_3 and C_2H_5).



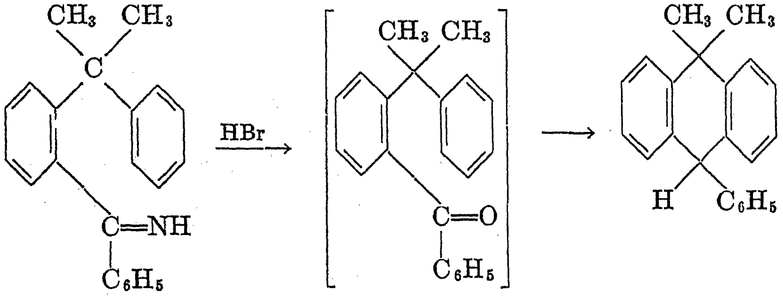
XXXIV XXXV

On the basis of their observation, the authors presented the following mechanism:

Mechanism of anthracene cyclization



Essentially the same mechanism was offered by Berliner (12) in a paper dealing in part with the same problem.



XXXVI XXXVII XXXVIII

In an effort to effect a cyclization yielding a 9,10-dihydroanthracene derivative whose structure could be demonstrated by synthesis, Bradsher and Smith (42) refluxed the imine XXXVI with hydrobromic acid. Despite the obvious inability of the expected intermediate (XXXVII) to enolize, cyclization was effected, yielding by concomitant reduction a compound which was shown to be 9,9-dimethyl-10-phenyl-9,10-dihydroanthracene (XXXVIII).

Recently, Berliner (13) has found the rates of cyclization of some o-(α-phenylethyl)phenones to be first order with respect to the ketone. He has presumed that the rate was determined by the attack of the carbonium ion on the benzene ring. The rate constants for the cyclization of the alkyl ketones are recorded in table 12. Berliner concluded that this decrease in rate with increasing size of the alkyl group up to the butyl ketone can best be attributed to the increase in electron-releasing character of the alkyl group concerned. This electron release results in an increase of electron density on the positive central carbon atom, resulting in a decrease in cyclization rate.

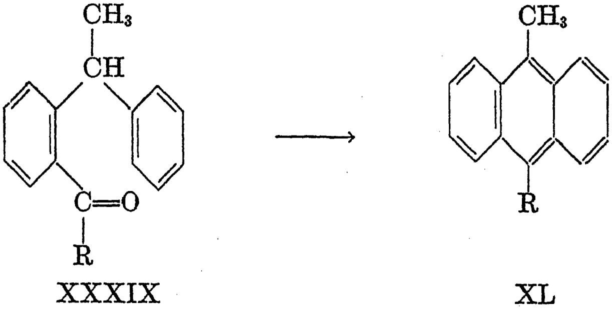


TABLE 12

KETONE R	K
	min.$^{-1}$ × *10*$^{-2}$
Methyl	4.6
Ethyl	1.8
n-Propyl	0.99
n-Butyl	0.35
n-Pentyl	0.36
n-Hexyl	0.36

V. SUMMARY

An effort has been made to correlate those cyclodehydration reactions which yield a new, fully aromatic ring through the acid-catalyzed attack of a ketonic or aldehydic carbonyl group on an aromatic nucleus. In each system discussed, the apparent limitations of the cyclization are pointed out, together with the effect of substituents on the ease of cyclization. The mechanism has been briefly considered.

VI. REFERENCES

(1) Autenrieth, W.: Ber. **24,** 159 (1891).
(2) Auwers, K. von, and Keil, G.: Ber. **36,** 1861 (1903).
(3) Auwers, K. von, and Keil, G.: Ber. **36,** 3902 (1903).
(4) Auwers, K. von, and Kraul, R.: Ber. **58,** 543 (1925).
(5) Auwers, K. von, and Möller, K.: J. prakt. Chem. **109,** 124 (1925).
(6) Axe, W.: J. Am. Chem. Soc. **61,** 1017 (1939).
(7) Axe, W., and Bailey, J.: J. Am. Chem. Soc. **60,** 3028 (1938).
(8) Axe, W., and Bailey, J.: J. Am. Chem. Soc. **61,** 2609 (1939).
(9) Bayer, F., and Co.: German patent 126,444; Chem. Zentr. **1902,** I, 78.
(10) Bayer, F., and Co.: German patent 239,544; Chem. Zentr. **1911,** II, 1396.
(11) Bergmann, E.: J. Org. Chem. **4,** 1 (1939).
(12) Berliner, E.: J. Am. Chem. Soc. **64,** 2894 (1942).
(13) Berliner, E.: J. Am. Chem. Soc. **66,** 533 (1944).
(14) Bernthsen, A.: Ann. **192,** 1 (1878).
(15) Bernthsen, A.: Ber. **15,** 3011 (1882).
(16) Bernthsen, A.: Ber. **16,** 767 (1883).
(17) Bernthsen, A., and Bender, F.: Ber. **16,** 1802 (1883).
(18) Besthorn, E., Bonzhaf, E., and Jaegle, G.: Ber. **27,** 3035 (1894).
(19) Besthorn, E., and Fischer, O.: Ber. **16,** 68 (1883).
(20) Besthorn, E., and Jaegle, G.: Ber. **27,** 907 (1894).
(20a) Bettzieche, F.: Z. physiol. Chem. **150,** 177 (1925).
(21) Beyer, C.: Ber. **20,** 1767 (1887).
(22) Bischler, A.: Ber. **25,** 2860 (1892).
(23) Bischler, A., and Fireman, P.: Ber. **26,** 1336 (1893).
(24) Borsche, W.: Ann. **377,** 70 (1910).
(25) Bougault, J.:Compt. rend. **159,** 745 (1915).
(26) Bradsher, C.: J. Am. Chem. Soc. **61,** 3131 (1939).
(27) Bradsher, C.: J. Am. Chem. Soc. **62,** 486 (1940).
(28) Bradsher, C.: J. Am. Chem. Soc. **62,** 1007 (1940).
(29) Bradsher, C.: J. Am. Chem. Soc. **64,** 1007 (1942).
(30) Bradsher, C., and Amore, S.: J. Am. Chem. Soc. **63,** 493 (1941).
(31) Bradsher, C., and Amore, S.: J. Am. Chem. Soc. **65,** 2016 (1943).
(32) Bradsher, C., and Amore, S.: J. Am. Chem. Soc. **66,** 1280 (1944).
(33) Bradsher, C., and Burhans, A.: J. Am. Chem. Soc. **62,** 3140 (1940).
(34) Bradsher, C., and Rapoport, L.: J. Am. Chem. Soc. **65,** 1646 (1943).
(35) Bradsher, C., and Rapoport, L.: J. Am. Chem. Soc. **66,** 1281 (1944).
(36) Bradsher, C., and Rapoport, L.: Unpublished work.
(37) Bradsher, C., and Rosher, L.: J. Am. Chem. Soc. **61,** 1524 (1939).
(38) Bradsher, C., and Schneider, A.: J. Am. Chem. Soc. **60,** 2960 (1938).
(39) Bradsher, C., and Smith, E.: Abstracts (page 20) of papers presented before the Division of Organic Chemistry at the 104th Meeting of the American Chemical Society, which was held in Memphis, Tennessee, April, 1942.
(40) Bradsher, C., and Smith, E.: J. Am. Chem. Soc. **65,** 451 (1943).
(41) Bradsher, C., and Smith, E.: J. Am. Chem. Soc. **65,** 854 (1943).
(42) Bradsher, C., and Smith, E.: J. Am. Chem. Soc. **65,** 1643 (1943).
(43) Bradsher, C., and Tess, R.: J. Am. Chem. Soc. **61,** 2184 (1939).
(44) Bradsher, C., and Wert, R.: J. Am. Chem. Soc. **62,** 2806 (1940).
(45) Bradsher, C., and Wissow, L.: J. Am. Chem. Soc. **65,** 2304 (1943).
(46) Bradsher, C., and Wissow, L.: Unpublished work.
(47) Braun, J. von, Gmelin, W., and Petzgold, A.: Ber. **57,** 382 (1924).
(48) Brown, W., and Bluestein, B.: J. Am. Chem. Soc. **62,** 3256 (1940).
(49) Bülow, C., and Issler, G.: Ber. **36,** 2447 (1903).
(50) Claisen, L., and Fischer, L.: Ber. **20,** 2191 (1881).

(51) Claisen, L., and Fischer, L.: Ber. **21**, 1135 (1882).
(52) Collet, A.: Bull. soc. chim. [3] **17**, 66 (1897).
(53) Combes, A.: Bull. soc. chim. [2] **49**, 89 (1888).
(54) Combes, A.: Compt. rend. **106**, 142 (1888).
(55) Carter, H., and Van Loon, E.: J. Am. Chem. Soc. **60**, 1077 (1938).
(56) Cook, J.: J. Chem. Soc. **1931**, 487.
(57) Cook, J.: Annual Reports on the Progress of Chemistry **39**, 178 (1942), The Chemical Society, London.
(58) Culmann, J.: Ber. **21**, 2595 (1888).
(59) Damman, K., and Gattermann, L.: Z. für farb. und text. Chem. **1**, 325 (1902); Chem. Zentr. **1902**, II, 368.
(60) Delisle, A.: Ann. **260**, 250 (1890).
(61) Delisle, A., and Schwalm, A.: Ber. **25**, 2980 (1892).
(62) Erlenmeyer, E., Jr., and Kunlin, J.: Ber. **35**, 384 (1902).
(63) Erlenmeyer, E., Jr., and Matter, O.: Ann. **337**, 271 (1904).
(64) Farben, I. G.: German patent 614,396; Chem. Zentr. **1935**, II, 2127.
(65) Fieser, L. F.: *Organic Reactions*, Vol. 1, p. 131. J. Wiley and Sons, Inc., New York (1944).
(66) Fieser, L. F., and Bradsher, C.: J. Am. Chem. Soc. **58**, 1738 (1936).
(67) Fieser, L. F., and Holmes, H.: J. Am. Chem. Soc. **58**, 2319 (1936).
(68) Fieser, L. F., and Holmes, H.: J. Am. Chem. Soc. **60**, 2548 (1938).
(69) Fischer, E.: Ann. **236**, 126 (1886).
(70) Fischer, E.: Ber. **26**, 764 (1893).
(71) Fischer, E., and Schmitt, T.: Ber. **21**, 1071 (1888).
(72) Fischer, E., and Schmitt, T.: Ber. **21**, 1811 (1888).
(73) Fischer, O.: Ber. **16**, 1820 (1883).
(74) Fischer, O., Schiebe, G., Merkel, P., and Müller, R.: J. prakt. Chem. **100**, 91 (1920).
(75) Friedländer, P., and Kunz, K.: Ber. **55**, 1597 (1922).
(76) Friedländer, P., and Schick, G.: Z. für farb. und text. Chem. **2**, 430 (1903); Chem. Zentr. **1904**, I, 101,
(77) Fries, K., Herring, H., Hemmicken, E., and Sichert, G.: Ann. **527**, 83 (1937).
(78) Fritsch, P.: Ber. **26**, 419 (1893).
(79) Fritsch, P.: Ann. **286**, 1 (1895).
(80) Geissman, T., and Tess, R.: J. Am. Chem. Soc. **62**, 514 (1940).
(81) Glenn, R., and Bailey, J.: J. Am. Chem. Soc. **61**, 2612 (1939).
(82) Glenn, R., and Bailey, J.: J. Am. Chem. Soc. **63**, 641 (1941).
(83) Graves, G., and Adams, R.: J. Am. Chem. Soc. **45**, 2439 (1923).
(84) Hantzsch, A.: Ber. **19**, 1290 (1886).
(85) Hantzsch, A., and Pfeiffer, G.: Ber. **19**, 1301 (1886).
(86) Haworth, R., Richardson, T., and Sheldrich, G.: J. Chem. Soc. **1935**, 1576.
(87) Haworth, R., and Sheldrich, G.: J. Chem. Soc. **1935**, 636.
(88) Haworth, R., and Woodcock, D.: J. Chem. Soc. **1938**, 809.
(89) Hell, C., and Bauer, H.: Ber. **37**, 872 (1904).
(90) Hell, C., and Cobin, H.: Ber. **37**, 866 (1904).
(91) Hesse, J.: Ber. **30**, 1438 (1897).
(92) Howell, W., and Robertson, A.: J. Chem. Soc. **1936**, 587.
(93) Ince, W.: Ann. **253**, 35 (1889).
(93a) Ingold, C.: Annual Reports on the Progress of Chemistry **25**, 135 (1938), The Chemical Society, London.
(94) Jacobsen, R., and Adams, R.: J. Am. Chem. Soc. **45**, 2455 (1923).
(95) Japp, F., and Murray, T.: Ber. **26**, 2638 (1893).
(96) Jensen, H., and Friedrich, M.: J. Am. Chem. Soc. **49**, 1049 (1927).
(97) Jensen, H., and Rethwisch, F.: J. Am. Chem. Soc. **50**, 1144 (1928).

(98) Johnson, W.: *Organic Reactions*, Vol. 2, p. 120. J. Wiley and Sons, Inc., New York (1944).
(99) Johnson, W., and Matthews, F.: J. Am. Chem. Soc. **66**, 210 (1944).
(100) Julian, P., and Pikl, J.: J. Am. Chem. Soc. **55**, 2105 (1933).
(101) Kliegl, A.: Ber. **38**, 84 (1905).
(102) Koenigs, W., and Jaegle, G.: Ber. **28**, 1046 (1895).
(103) Koenigs, W., and Mengel, A.: Ber. **37**, 1322 (1904).
(104) Lempert, H., and Robinson, R.: J. Chem. Soc. **1934**, 1419.
(105) Lions, F.: J. Proc. Roy. Soc. N. S. Wales **63**, 159 (1930); Chem. Abstracts **24**, 5300 (1930).
(105a) Lions, F., Perkin, W., and Robinson, R.: J. Chem. Soc. **127**, 1158 (1925).
(106) Marckwald, W., and Schmidt, C.: Ann. **274**, 367 (1893).
(107) Mayer, F., and Freund, W.: Ber. **55**, 2049 (1922).
(108) Mayer, F., and Levis, I.: Ber. **52**, 1641 (1919).
(109) Mayer, F., and Stein, B.: Ber. **50**, 1306 (1917).
(110) Meerwein, H.: Ann. **405**, 129, 173 (1914).
(111) Meisenheimer, J.: Ber. **57**, 1744 (1924).
(112) Mitter, P., and De, S.: J. Indian Chem. Soc. **16**, 35 (1939).
(113) Möhlau, R.: Ber. **14**, 171 (1881).
(114) Möhlau, R.: Ber. **15**, 2480 (1882).
(115) Möhlau, R.: Ber. **18**, 163 (1885).
(116) Möhlau, R.: Ber. **21**, 510 (1888).
(117) Murray, R., and Turner, E.: J. Chem. Soc. **1934**, 856.
(118) Nencki, M., and Berlinerblau, J.: Ber. **20**, 753 Ref. (1887).
(119) Ohmaki, T.: J. Pharm. Soc. Japan **58**, 4 (1938).
(120) von Pechmann, A.: Ber. **16**, 516 (1883).
(121) Perkin, W., and Robinson, R.: J. Chem. Soc. **105**, 2376 (1914).
(122) Pictet, A.: Ber. **19**, 1063 (1886).
(123) Pictet, A., and Duparc, L.: Ber. **20**, 3415 (1887).
(124) Pomeranz, P.: Monatsh. **14**, 116 (1893).
(125) Pomeranz, P.: Monatsh. **15**, 299 (1894).
(126) Pomeranz, P.: Monatsh. **15**, 739 (1894).
(127) Pomeranz, P.: Monatsh. **18**, 1 (1897).
(128) Rinkes, I.: Rec. trav. chim. **39**, 200 (1920).
(129) Roberts, E., and Turner, E.: J. Chem. Soc. **1927**, 1832.
(130) Robinson, R., and Tomlinson, M.: J. Chem. Soc. **1934**, 1524.
(131) Rugheimer, L., and Schön, P.: Ber. **42**, 2374 (1909).
(132) Schenk, L., and Bailey, J.: J. Am. Chem. Soc. **61**, 2613 (1939).
(133) Schönberg, A., and Warren, F.: J. Chem. Soc. **1939**, 1838.
(134) Späth, E.: Monatsh. **33**, 1029 (1912).
(135) Staedel, W.: Ber. **21**, 2196 (1888).
(136) Staub, P.: Helv. Chim. Acta **5**, 888 (1922).
(137) Stoermer, R.: Ber. **28**, 1253 (1895).
(138) Stoermer, R.: Ann. **312**, 237 (1900).
(139) Stoermer, R., and Atenstädt, P.: Ber. **35**, 3560 (1902).
(140) Stoermer, R., and Wehln, R.: Ber. **35**, 3549 (1902).
(141) Stollé, R.: J. prakt. Chem. **105**, 137 (1922).
(142) Thielepape, E.: Ber. **55**, 127 (1922).
(143) Tyson, F.: J. Am. Chem. Soc. **61**, 183 (1939).
(144) Ullmann, F., and Broido, J.: Ber. **39**, 356 (1906).
(145) Ullmann, F., and Ernst, H.: Ber. **39**, 298 (1906).
(146) Verkade, P., and Janetzky, E.: Rec. trav. chim. **62**, 763 (1943).
(147) Verkade, P., and Janetsky, E.: Rec. trav. chim. **62**, 775 (1943).
(148) Vladesco, D.: Bull. soc. chim. [3] **6**, 807 (1891).

(149) WOLFF, L.: Ber. **21,** 123 (1888).
(150) WALSH, G., AND WEIZMANN, C.: J. Chem. Soc. **97,** 685 (1910).
(151) WEITZENBOCK, R.: Monatsh. **34,** 193 (1913).
(152) WERNER, A., AND GROB, A.: Ber. **37,** 2887 (1904).
(152a) WOODWARD, R., AND DOERING, W.: J. Am. Chem. Soc. **67,** 860 (1945).
(153) ZINCKE, T., AND BREUER, A.: Ann. **226,** 23 (1884).
(154) ZINCKE, T.: Ann. **240,** 137 (1887).

THE ISOLATION AND SYNTHESIS OF THE NATURALLY OCCURRING α-AMINO ACIDS

RICHARD J. BLOCK

The Department of Physiology and Biochemistry, New York Medical College, Flower and Fifth Avenue Hospitals, New York City, and Cooper Road, Scarsdale, New York

Received September 13, 1945

CONTENTS

I. Introduction 504
II. Isolation from natural sources 504
  A. Arginine, histidine, and lysine 505
    1. Electrolytic separation 505
    2. Selective adsorption 506
    3. Individual separation of arginine, histidine, and lysine 507
      (a) Arginine *via* arginine flavianate 507
      (b) Arginine *via* the benzylidene derivative 508
      (c) Separation of histidine by means of metallic derivatives 508
      (d) Isolation of histidine by aromatic sulfonic acids 508
      (e) Lysine picrate 509
      (f) Benzoyllysine copper 509
  B. Glutamic acid and aspartic acid 509
    1. Precipitation as the calcium or barium salts 509
    2. Electrolytic separation 509
    3. Selective adsorption 509
  C. Glutamic acid, glutamine, aspartic acid, and asparagine 510
    1. Glutamic acid 510
    2. Glutamine 511
    3. Aspartic acid 511
    4. Asparagine 511
  D. Isolation of cystine and cysteine 511
    1. Isoelectric precipitation 511
    2. Precipitation of cuprous cysteine mercaptide 511
  E. Tyrosine 511
  F. Tryptophan 512
  G. Leucine, isoleucine, and valine 512
  H. Miscellaneous 512
    1. Alanine 512
    2. Diiodotyrosine 512
    3. Glycine 512
    4. Hydroxylysine 513
    5. Hydroxyproline 513
    6. Methionine 513
    7. Phenylalanine 513
    8. Proline 513
    9. Serine 513
  I. General isolation methods 513
III. Synthesis 514
  A. Amination of α-halogen acids 514
    1. From monocarboxylic acids 514
      (a) Preparation of glycine 514

(b) Synthesis of alanine, valine, leucine, norleucine, etc. 514
(c) Synthesis of lysine, proline, and other amino acids 515
2. From substituted malonic acids 517
(a) Preparation of valine, isoleucine, norleucine, and phenylalanine 517
(b) Preparation of proline and hydroxyproline 517
(c) Synthesis of histidine 518
(d) Preparation of methionine and homocystine 519
3. From potassium (sodium) phthalimide 520
(a) Gabriel synthesis 520
4. Miscellaneous methods 521
B. Hydrolysis of aminocyanohydrins (Strecker synthesis) 523
1. Alanine 523
2. Glycine 524
3. Leucine, valine, isoleucine, and phenylalanine 524
4. Glutamic acid 524
5. Methionine 525
6. Serine 526
C. Condensation of an aldehyde with an active methylene group: The Perkin reaction (Erlenmeyer's synthesis) 526
1. Phenylalanine and tyrosine 526
(a) By condensation with hippuric acid 526
(b) By condensation with hydantoins 527
(c) By condensation with thiohydantoin 528
(d) By condensation with diketopiperazine 528
(e) By condensation with rhodanine 529
(f) By condensation with acetylglycine 529
2. Tryptophan 529
(a) By condensation with hippuric acid 529
(b) By condensation with hydantoin 531
3. Thyroxine 532
(a) By condensation with benzoylglycine 532
4. Histidine 533
(a) By azlactone synthesis 533
5. Lysine 533
(a) By condensation with malonic acid 533
D. Reduction of α-keto groups and their derivatives 533
1. Reduction of α-keto groups in the presence of ammonia: alanine, leucine, isoleucine, glutamic acid, etc. 533
2. Reduction of phenylhydrazones: alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, etc. 534
3. Reduction of oximes 535
(a) Preparation of oxime with hydroxylamine: aspartic acid, asparagine, glutamic acid, alanine, phenylalanine, tyrosine, etc. 535
(b) Preparation of oxime by action of nitrite on substituted malonic acid or ester: lysine, leucine, phenylalanine 536
(c) Preparation of oxime by action of nitrite on substituted acetoacetic ester: methionine, threonine, hydroxyproline, aspartic acid, glutamic acid, leucine, isoleucine, etc. 536
(d) Syntheses from α-oximino-γ-butyrolactone 537
E. Alkylation of aminomalonic acids 538
1. Phthalimidomalonic ester 538
(a) Lysine 538
(b) Phenylalanine and tyrosine 539

    - (c) Proline and hydroxyproline 540
    - (d) Serine and β-hydroxynorvaline 541
    - (e) Methionine and cystine 541
    - (f) Glutamic acid and aspartic acid 542
  - 2. Aminomalonic ester 542
    - (a) Proline 542
    - (b) Glycine, leucine, and phenylalanine 543
  - 3. Benzoylaminomalonic ester 543
    - (a) Glutamic acid, aspartic acid, glycine, alanine, leucine, valine, and phenylalanine 543
  - 4. Acetylaminomalonic ester 544
    - (a) Leucine, norleucine, and phenylalanine 544
    - (b) Histidine 544
    - (c) Tryptophan 545
  - 5. Acetylaminocyanoacetic ester 545
    - (a) Valine, methionine, phenylalanine, tryptophan, histidine, etc 545
- F. Azide synthesis 546
  - 1. Potassium ethyl malonate: Curtius reaction 546
    - (a) Alanine, valine, leucine, and glycine 546
    - (b) Phenylalanine 546
  - 2. Cyanoacetic ester: Curtius reaction 547
    - (a) Glycine 547
    - (b) Leucine, valine, norleucine, tyrosine, and phenylalanine 547
  - 3. Cyanoacetic ester: Hofmann rearrangement 548
  - 4. Hydrazoic acid: Schmidt reaction 548
    - (a) Glycine, phenylalanine, and aspartic acid 548
    - (b) Lysine 549
    - (c) Phenylalanine 549
- G. Miscellaneous 549
  - 1. Alanine and glycine 549
    - (a) Glycine from cyanide 549
    - (b) Glycine and alanine by metathesis 550
    - (c) Glycine and alanine by oxidation of amino alcohols 551
  - 2. Aspartic and glutamic acids 551
    - (a) Aspartic acid from maleic or fumaric acid 551
    - (b) Glutamic acid by Michael condensation 552
    - (c) Glutamine and asparagine 552
  - 3. Serine and threonine 553
    - (a) Serine from ethyl hippurate 553
    - (b) Serine and threonine by the addition of mercury salts to α,β-olefincarboxylic esters 553
    - (c) Serine and threonine by halogenation of olefinic acids 554
  - 4. Tyrosine, halogenated tyrosines, and thyroxine 554
    - (a) Tyrosine 554
    - (b) Iodogorgoic acid, dibromotyrosine, and dichlorotyrosine 554
    - (c) Thyroxine from diiodotyrosine 555
  - 5. Proline 556
    - (a) Proline from pyrrolidonecarboxylic acid 556
    - (b) Proline from pyrrole 556
  - 6. Arginine and citrulline 557
    - (a) Arginine from ornithine 557
    - (b) Citrulline from arginine or ornithine 557
  - 7. Cysteine and methionine 557

(a) Cysteine from serine.. 557
(b) Methionine from ethyl chloroethylhippurate........................ 557
8. β-Alanine... 557
H. General.. 558
IV. References... 558

I. INTRODUCTION

Within the past decade, interest in the α-amino acids has greatly quickened and has turned from a concern primarily with the structure of proteins to the more mundane consideration of amino acids as nutritive agents. This change of view has shifted interest to the large-scale preparation of amino acids either by isolation from proteins or other natural sources or by organic synthetic methods. It is hoped in this review to describe the methods which are being used or which show promise of use for the preparation of the naturally occurring α-amino acids for large-scale production. Some of the procedures employed in the past for proof of structure may be mentioned briefly while others, as yet limited to small-scale laboratory preparations, will be described in greater detail where the author is of the opinion that they offer future possibilities.

Table 1 lists the amino acids which will be considered in this review. The great similarity in chemical composition, coupled with high melting points, precludes the separation of most of the naturally occurring amino acids by the conventional methods of organic chemistry—i.e., fractional distillation and crystallization. As will be seen below, successful separation has been found worthwhile in a few cases but it appears that, for practical purposes, reliance on synthetic methods will be the choice of the future for the majority of amino acids.

II. ISOLATION FROM NATURAL SOURCES

The literature pertaining to the isolation of amino acids by the elaborate methods whose primary purpose concerns their quantitative determination in proteins, etc., has been recently summarized by Block and Bolling (48) and will not be considered here.

Before an amino acid can be separated from a protein or protein concentrate, it is necessary to hydrolyze the protein into its constituent parts. This can be accomplished by heating with aqueous acid or alkali or by digestion with proteolytic enzymes. The conditions employed can be subjected to wide variations, depending on the starting material and on the particular amino acid desired. In common practice, 20 to 50 per cent aqueous sulfuric acid or 20 to 38 per cent hydrochloric acid at atmospheric or preferably at increased pressure is usually employed (*cf.* 41, 42, 43, 44, 45, 48, 50 for details). The excess mineral acid is then removed from the resulting amino acid mixture by the addition of an aqueous suspension of calcium hydroxide in the case of sulfuric acid, while distillation *in vacuo* is used to remove excess hydrochloric acid. Anion-exchange resins can also be advantageously employed in this connection (*cf.* 42, 44). In many cases, it is worthwhile to adsorb humin and other impurities by means of activated carbon. Alkaline and enzymic hydrolysis are employed only in special cases, which will be given below.

A. ARGININE, HISTIDINE, AND LYSINE

The presence of extra nitrogen atoms in the molecules of these three amino acids has permitted their separation from the other constituents of protein hydrolysates.

TABLE 1

AMINO ACID	FORMULA	OPTICAL ROTATION	MOLECULAR WEIGHT	CHEMICAL COMPOSITION					MELTING POINT
				C	H	N	O	S or I	
									°C.
Alanine	$C_5H_7O_2N$	+	89.07	40.42	7.93	15.73	35.92		297
Arginine	$C_6H_{14}O_2N_4$	+	174.14	41.35	8.10	32.18	18.37		220 (as HCl)
Aspartic acid	$C_4H_7O_4N$	−	133.07	36.08	5.31	10.53	48.11		251
Asparagine	$C_4H_8O_3N_2$	−	132.08	36.36	6.11	21.22	36.36		226–227
Citrulline	$C_6H_{13}O_3N_3$		175.12	41.11	7.48	23.99	27.41		
Cystine	$C_6H_{12}O_4N_2S_2$	−	240.23	29.97	5.03	11.66	26.64	26.69	256–258
Diiodotyrosine	$C_9H_9O_3NI_2$	−	432.91	24.97	2.10	3.24	11.09	58.63	
Glutamine	$C_5H_{10}O_3N_2$	−	146.10	41.07	6.90	19.18	32.85		254–256 (*dl*)
Glutamic acid	$C_5H_9O_4N$	+	147.08	40.80	6.17	9.50	43.51		197–198
Glycine	$C_2H_5O_2N$		75.05	31.98	6.71	18.67	42.64		225–330
Histidine	$C_6H_9O_2N_3$	−	155.09	46.42	5.85	27.10	20.63		270
Hydroxylysine	$C_6H_{14}O_3N_2$		162.13	44.08	8.70	17.28	29.61		225 (as picrate)
Hydroxyproline	$C_5H_9O_3N$	−	131.08	45.77	6.92	10.69	36.62		270
Isoleucine	$C_6H_{13}O_2N$	+	131.11	54.92	9.99	10.69	24.41		280
Leucine	$C_6H_{13}O_2N$	−	131.11	54.92	9.99	10.69	24.41		293–295
Lysine	$C_6H_{14}0_2N$	+	146.13	49.27	9.66	19.17	21.90		263–264 (as HCl)
Methionine	$C_5H_{11}O_2NS$	−	149.15	40.23	7.43	9.39	21.45	21.50	283 (uncorrected)
Phenylalanine	$C_9H_{11}O_2N$	−	165.09	65.41	6.72	8.49	19.38		283
Proline	$C_5H_9O_2N$	−	115.08	52.14	7.88	12.17	27.81		220–222
Serine	$C_3H_7O_3N$	−	105.08	34.27	6.72	13.33	45.68		228
Threonine	$C_4H_9O_3N$	−	119.08	40.31	7.62	11.74	40.31		251–253
Thyroxine	$C_{15}H_{11}O_4NI_4$	−	776.82	23.17	1.43	1.80	8.24	65.35	235–236
Tryptophan	$C_{11}H_{12}O_2N_2$	−	204.11	64.67	5.93	13.72	15.68		289
Tyrosine	$C_9H_{11}O_3N$	−	181.09	59.64	6.12	7.74	26.50		314–318
Valine	$C_5H_{11}O_2N$	+	117.10	51.24	9.47	11.96	27.33		315

1. *Electrolytic separation*

In 1912, Ikeda and Suzuki (168) obtained a patent for separating arginine, histidine, and lysine based on placing a protein hydrolysate, from which the greater part of the mineral acid had been removed, in a three-compartment electrodialysis apparatus and subjecting it to electrolysis. Electrolytic separation of arginine, histidine, and lysine has been further investigated by Foster and Schmidt (133), Cox, King, and Berg (82), and others (*cf.* 48). In all cases, the migration of the basic amino acids to the cathode compartment is utilized. The more acidic amino acids are excluded because of the alkalinity of the cathode compartment. Figure 1 is a drawing of the apparatus used by Cox *et al.* (82), employing graphite electrodes.

2. *Selective adsorption*

Sadikov *et al.* (260) reported that permutit removed 93 per cent of the diamino acids and 50 per cent of the monoamino acids from a casein hydrolysate. The amino acids could then be eluted with 30 per cent sulfuric acid. Turba (317) used bleaching earths (Filtrol, Floridin, etc.) to separate arginine, histidine, and lysine from other amino acids and from one another. However, his published work has been designed for their quantitative separation rather than for large-scale preparation. Turba uses 2500 mg. of bleaching earth to separate 25 mg. of lysine. Furthermore, it is not clear whether the adsorbent can be used over and over again.

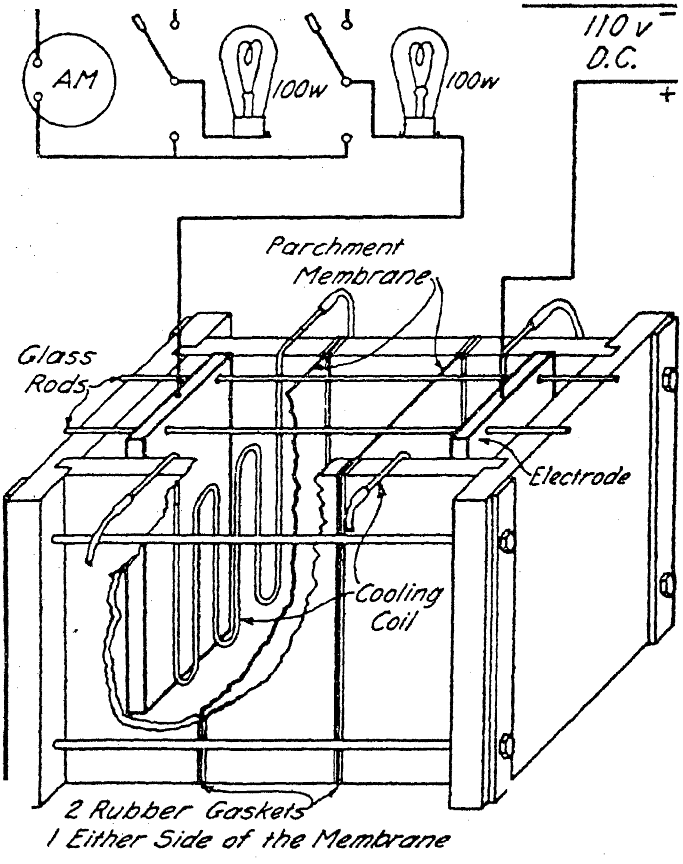


FIG. 1. Electrolytic separation of amino acids (J. Biol. Chem. 81, 755–64 (1929))

Block (41, 42, 43, 45) has used the commercially available cation-exchange resins (polymerized sulfonated phenol–formaldehyde resins), such as Amberlite

IR-1, Amberlite IR-100, Duolite C-1, Duolite C-3, Amberlite XE-17, Ionac C-284, and others, for the concentration of polyamino acids with respect to monoamino acids. Thus, a single passage of a blood hydrolysate, prepared from 50 g. of blood meal (80 per cent protein), through a 50-g. column of cation-exchange resin in the hydrogen cycle resulted in the complete adsorption of arginine, histidine, and lysine and a partial adsorption of some of the monoamino acids. After washing the column with water, the adsorbed amino acids were eluted with constant-boiling hydrochloric acid or exchanged against 4 per cent aqueous ammonia. If desired, histidine and monoamino acids may be removed from the resin by elution with aqueous pyridine before elution of arginine and lysine with dilute ammonia. Whereas the original blood hydrolysate contained approximately twice as much monoamino as polyamino acids, the elutriates contained twice as much diamino as monoamino acids. Other sources of amino acids, such as soybean meal, fish meal, shark meat, etc., can be used with equal facility. The ion exchanger after acid elution is ready for the next cycle without further treatment; if ammonia exchange is used to recover the polyamino acids, it is better, although not necessary, to convert the exchanger to the hydrogen form before the next run. The life of these exchangers exceeds 500 cycles by laboratory test.

Block has also found (42, 43, 45) that not all cation-exchange materials are suitable for the separation of polyamino acids; thus, the sulfonated coals, which are utilized extensively for water softening and other purposes, have not been found operative under the above-described experimental conditions.

3. Individual separation of arginine, histidine, and lysine

Although both the electrolytic and the adsorption methods can be used to separate the three polyamino acids from one another, for large-scale preparation of arginine, histidine, and lysine it seems preferable first to employ one of the group separations given above with one or more of the methods designed for isolation of a specific polyamino acid to be given below.

(a) Arginine *via* arginine flavianate

Kossel and Gross (190, 191) observed that arginine could be readily and practically quantitatively precipitated from acid hydrolysates of arginine-rich proteins, such as gelatin, by the addition of a considerable excess of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid). The resulting arginine mono(or di)flavianate was then decomposed by dissolving in dilute ammonium hydroxide and precipitating the flavianic acid as the barium salt with baryta. Arginine carbonate was isolated from the filtrate after removal of the excess barium with carbon dioxide (*cf.* 166). An alternative method was to decompose the finely powdered arginine flavianate with hot 33 per cent sulfuric acid, cool, filter off the flavianic acid, and isolate arginine carbonate after removal of the sulfuric acid with baryta. Pratt (249) extracted the flavianic acid from sulfuric acid-decomposed arginine flavianate with butyl alcohol and subsequently isolated arginine carbonate according to Kossel and Gross (191). Cox (80) modified the Kossel methods by using hydrochloric acid for hydrolysis and throughout the procedure.

He decomposed the arginine flavianate with hot concentrated hydrochloric acid, cooled the solution to crystallize out flavianic acid, and precipitated arginine dihydrochloride from the concentrated filtrate with alcohol. The dihydrochloride was then converted into arginine monohydrochloride by the addition of aniline. Aniline hydrochloride is soluble in alcohol, while arginine monohydrochloride is not. Block (46) decomposed arginine flavianate by stirring an aqueous suspension with an anion–exchange resin (Amberlite IR–4B).

Arginine flavianate can be recrystallized from dilute ammonium hydroxide by acidification with hydrochloric or acetic acid (*cf.* 48).

(b) Arginine *via* the benzylidene derivative

Bergmann and Zervas (32, 33) found that benzaldehyde will condense with arginine in strongly alkaline solution (barium hydroxide or sodium hydroxide) at 0–5°C. to yield the insoluble sodium or barium benzylidenearginine. The salt is then washed with ice water, and dried with alcohol and ether. The benzylidenearginine is decomposed by heating with 5 *N* hydrochloric or nitric acid and, after extraction of the benzaldehyde with ether, arginine is isolated as the hydrochloride or nitrate.

(c) Separation of histidine by means of metallic derivatives

Kossel (189, 192) in 1898 utilized the insolubility of histidine mercuric chloride or histidine silver at neutrality as the means of separating this amino acid from protein hydrolysates. Since that time Vickery and Leavenworth (322), Jones (172), and others have used either or both of these salts, with modifications in detail (*cf.* 48, 134, 142, 152, 172, etc.), for the isolation of histidine from hemoglobin or blood meal. Kapfhammer and Spörer (174) found that histidine was precipitated along with the other heterocyclic amino acids, proline and hydroxyproline, by Reinecke acid [4-tetrarhodanato-2-amminochromic acid] and suggested that use of this reagent, followed by precipitation with mercuric chloride at pH 7, would be a good way of preparing histidine. However, Reinecke acid does not appear to offer any advantage over the original Kossel methods.

(d) Isolation of histidine by aromatic sulfonic acids

The successful application by Kossel and Gross (191) of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid) for the separation of arginine prompted Bergmann and his associates (92, 301) to study the specific precipitability of other aromatic sulfonic acids. From a large number of these, Vickery (321) found that the easily prepared 3,4-dichlorobenzenesulfonic acid was an excellent precipitant for histidine. When blood cells are hydrolyzed with hydrochloric acid and the excess mineral acid is removed by repeated concentration *in vacuo*, histidine can be readily isolated from the decolorized hydrolysate by the addition of 5–6 moles of 3,4-dichlorobenzenesulfonic acid. The resulting histidinedisulfonate is readily recrystallized from boiling water. Histidine can then be isolated from the pure salt by precipitation of the 3,4-dichlorobenzenesulfonic acid with barium hydroxide. The excess barium hydroxide is removed with carbon dioxide, and histidine is isolated as the free base from the concentrated aqueous solution.

(e) Lysine picrate

Up to 1939, lysine was only isolated from protein hydrolysates by precipitation with the costly phospho-24-tungstic acid (*cf.* 48), followed by formation of lysine picrate. In that year, Rice (257) in W. C. Rose's laboratory reported that lysine picrate could be prepared directly from blood hydrolysates from which the excess sulfuric acid had been previously removed by precipitation with cream of lime to pH 6. The resulting lysine picrate, contaminated with histidine and other picrates, was purified by repeated crystallization from hot water. The lysine picrate was then decomposed by hot hydrochloric acid, the picric acid was removed by crystallization and with charcoal, and the lysine was isolated directly as the dihydrochloride or, after treatment with pyridine, as lysine monohydrochloride (*cf.* Section II,A,3,(a) above).

Unfortunately, this simple procedure does not appear to be generally applicable to crude commercial protein hydrolysates (46, 197, 257) but it gives excellent results when applied to polyamino acid concentrates prepared by ion-exchange adsorption (*cf.* Section II,A,2, above).

(f) Benzoyllysine copper

Kurtz (197) evolved a novel method for preparing lysine. The amino acids from a neutralized protein hydrolysate (pH 6) are converted into their copper salts by boiling with excess cupric carbonate. The excess cupric carbonate is filtered off, and the clear blue filtrate is treated with benzoyl chloride at 0°C. The ε-benzoyllysine copper which precipitates is washed with ice water and alcohol and dried in air. The copper is removed by hydrogen sulfide, and ε-benzoyllysine is allowed to crystallize from aqueous solution. Lysine is recovered from the benzoyl derivative following hydrolysis with 8 *M* hydrochloric acid for 10 hr.

B. GLUTAMIC ACID AND ASPARTIC ACID

1. Precipitation as the calcium or barium salts

The dicarboxylic amino acids can be precipitated from protein hydrolysates as the calcium or barium salts from strongly alkaline solution by the addition of 5–10 volumes of ethanol. Details of this procedure, which was first used as a quantitative method by Ritthausen and by Foreman, are given in reference 48.

2. Electrolytic separation

Foster and Schmidt (133, 168) also suggested that the dicarboxylic amino acids could be concentrated in the anodic compartment of the three-cell electrodialysis apparatus described in Section II,A,1. This suggestion has apparently not been developed further.

3. Selective adsorption

Turba and Richter (318; *cf.* 48) found that glutamic and aspartic acids are quantitatively adsorbed by Brockmann's standardized aluminum oxide. Glutamic acid is then eluted with *N* acetate–acetic acid buffer, after which the aspartic acid is removed with dilute alkali. It requires 5000 mg. of aluminum oxide to adsorb 10 to 15 mg. of

A superior analytical and preparative method has been devised by Cannan and Kibrick (67, 179). This consists in adsorbing the dicarboxylic amino acids on one of the polyamine phenol–formaldehyde resins such as Amberlite IR-4, Duolite A-3, De-Acidite, etc. Cannan (67) has described the conditions ensuing when an acidic protein hydrolysate is treated with sufficient anion-exchange resin to raise the pH to 6–7.

> "The neutral amino acids will be present almost entirely in the form of dipolar ions with zero net charge, the acidic amino acids in the form of monoanions, and the ammonia and the basic amino acids (except histidine) in the form of monocations. The histidine will be partially cationic and partially dipolar. The chief anions present will, therefore, be those of the hydrochloric acid and of the dicarboxylic amino acids; and it is these alone which should be adsorbed by the resin. Let *a*, *b*, and *c* represent the equivalents of acidic amino acids, bases (basic amino acids and ammonia), and chloride respectively in the original hydrolysate. Then, if α is the fraction of the total anions which is bound by the resin, and if α_a and α_c are the fractions of the total dicarboxylic amino acids and of chloride respectively which are bound, electrical neutrality in the solution requires that
>
> $$a + c - b = (a + c)\alpha = a\alpha_a + c\alpha_c$$
>
> $$\therefore\ \alpha = 1 - \frac{b}{a + c} \quad \text{"}$$

The α is determined by the ratio of bases to acids. Therefore, for complete removal of the dicarboxylic amino acids, hydrochloric acid must be present in very large excess relative to the bases, or the greater part of the bases must be removed from solution prior to treatment with the resin. In fact, the effluent from the cation adsorption of the basic amino acids has proved an excellent source for the adsorption of the dicarboxylic acids (unpublished experiments of the author and G. P. Taylor, 1941) (*cf.* Section II,A,2).

C. GLUTAMIC ACID, GLUTAMINE, ASPARTIC ACID, AND ASPARAGINE

1. *Glutamic acid*

Probably over 1,000,000 lb. of glutamic acid are prepared per year in the United States for use in the form of monosodium glutamate as a condiment, especially in soups. This is usually prepared from hydrolysates of wheat gluten, corn gluten, casein, soy bean proteins, or beet sugar wastes. However, wheat gluten is by far the largest source.

The protein is hydrolyzed with concentrated hydrochloric acid under pressure for several hours, the black hydrolysate is then decolorized with carbon, and the yellow-brown filtrate is concentrated to a small volume to permit the crystallization of glutamic acid hydrochloride. The glutamic acid hydrochloride is removed, dissolved in water, and the pH is adjusted to 3.2–3.3, whereupon glutamic acid crystallizes. The amino acid is then neutralized with sodium hydroxide to form monosodium glutamate ($C_5H_8O_4NNa$), which is recovered by crystallization. The monosodium glutamate is approximately 99 per cent pure. There are numerous publications and patents embodying minor modifications of the above method, a few of which are given (12, 24, 38, 79, 181, 205, 281, 342).

Apparently the most economical procedure for the preparation of monosodium glutamate is its isolation from the beet sugar by-product known as Steffen house waste (227). This dilute waste liquid is concentrated to a fairly high specific gravity and subjected to mild alkaline hydrolysis to liberate the glutamic acid. Further steps are similar to those employed for the isolation of monosodium glutamate from wheat gluten hydrolysates.

2. Glutamine

The glutamine present in beet juice is precipitated by mercuric nitrate at pH 7. After decomposition of the precipitate with hydrogen sulfide and sulfuric acid, glutamine is crystallized from the inorganic ion-free solution with the aid of alcohol (323).

3. Aspartic acid

This amino acid is best isolated, as the copper salt, from the dicarboxylic amino acid fraction prepared by one of the methods given in Section II,B, after precipitation of glutamic acid as the free base or the hydrochloride (*cf.* 48).

4. Asparagine

White lupin seedlings are extracted with water and the soluble proteins are removed. Asparagine crystallizes out of the aqueous solution on concentration *in vacuo* (248, 324).

D. ISOLATION OF CYSTINE AND CYSTEINE

1. Isoelectric precipitation

A cystine-rich protein, such as horse hair, rabbit fur, wool scraps, etc., is hydrolyzed for several hours with hydrochloric acid. The humin is removed by filtration and activated carbon. Cystine is then precipitated by neutralization of the light yellow hydrolysate with sodium hydroxide, ammonium hydroxide, or sodium acetate to pH 4.5–4.8. The crude cystine so obtained is recrystallized from hot dilute hydrochloric acid or hydrochloric acid–acetic acid, followed by neutralization to pH 4.5–4.8 with ammonium hydroxide (48, 131, 237, 238, 270, 330).

2. Precipitation of cuprous cysteine mercaptide

Lucas and Beveridge (214), using the observation of Hopkins and others (*cf.* 48) that cysteine is quantitatively precipitated by cuprous ions, found that when a protein hydrolysate containing cystine is treated with a suspension of cuprous oxide at pH 5, a quantitative precipitation of $CuSCH_2CHNH_2COOH$ results. The copper is then removed with hydrogen sulfide and cysteine hydrochloride is isolated.

Cystine and cysteine are prepared in commercial quantities by these general procedures and are being used in increasing quantities in the food (41, 49) and cosmetic industries.

E. TYROSINE

Tyrosine is usually obtained as by-product in the manufacture of both cystine and glutamic acid. It is very insoluble at its isoelectric point, *ca.* pH 5.7, a

F. TRYPTOPHAN

Tryptophan is best prepared by synthesis, but for the isolation of the naturally occurring form, a protein, preferably lactalbumin or blood fibrin, is digested for several weeks with commercial pancreatin at pH 8 (sodium bicarbonate) in the presence of toluene or chloroform as preservatives (81). The tryptophan is precipitated with mercuric sulfate in 10 per cent sulfuric acid (48, 81). After decomposition of the mercury precipitate with hydrogen sulfide, the tryptophan is extracted with butyl alcohol (86) and crystallized from the solvent (*cf.* 262, 327).

G. LEUCINE, ISOLEUCINE, AND VALINE

These three amino acids are also obtained as by-products in the preparation of glutamic acid and cystine. Most of the commercial "leucine" contains considerable quantities of isoleucine, valine, and even methionine. Barnett (22, 23) isolated leucine as dileucine hydrochloride from a neutralized casein hydrolysate at pH 1.7–2.8 in the presence of a large excess of sodium chloride, while Stein *et al.* (301) used 2-bromotoluene-5-sulfonic acid to precipitate leucine from a leucine-rich, isoleucine-poor protein hydrolysate (hemoglobin).

A mixture consisting of approximately 50 per cent leucine and 50 per cent isoleucine is obtained as a by-product in the manufacture of monosodium glutamate from beet sugar waste. Isoleucine can be separated from leucine by making use of Ehrlich's observation that its copper salt is considerably more soluble in methanol than that of leucine (*cf.* 48, 102). Specific organic reagents may also be developed for this purpose. Town (314) reported that it is possible to separate leucine and valine by fractional precipitation.

H. MISCELLANEOUS

1. Alanine

This amino acid has been obtained from silk fibroin hydrolysates after preliminary removal of glycine as the insoluble azobenzene-*p*-sulfonic acid salt (301) and as the insoluble alanine calcium picrate (124).

2. Diiodotyrosine

Iodogorgoic acid was obtained by Wheeler and Mendel (335) from hydrolysates of the common bath sponge by precipitation with silver nitrate and ammonium hydroxide, followed by phospho-24-tungstic acid.

3. Glycine

Glycine is readily isolated from glycine-rich protein hydrolysates by precipitation of the ester hydrochloride (*cf.* 2, 48). It also forms an insoluble salt with calcium picrate $NH_2CH_2COOH \cdot [C_6H_2(NO_2)_3O]Ca \cdot 2H_2O$ (124) and with nitranilic acid (2,5-dihydroxy-3,6-dinitro-*p*-benzoquinone) (313). Inorganic ions, ammonia, and basic amino acids also form insoluble nitranilates (313; *cf.* 48).

4. Hydroxylysine

This amino acid has been isolated in small quantities from gelatin and isinglass by Schryver *et al.* (9, 28) and Van Slyke *et al.* (320). The method used is based upon the Kossel–Kutscher procedure for lysine (*cf.* 48), followed by fractional crystallization of lysine and hydroxylysine picrates.

5. Hydroxyproline

This amino acid is precipitated along with proline by Reinecke acid (4-tetrarhodanato-2-amminochromic acid) (174). Hydroxyproline is not soluble in absolute ethanol, which dissolves proline (*cf.* 184, 215, 312).

6. Methionine

Methionine is isolated from protein hydrolysates only with considerable difficulty. Pirie (246) used butyl alcohol extraction, followed by precipitation with mercuric acetate. Approximately 30 per cent of the methionine present in casein was obtained by this method.

7. Phenylalanine

This compound has been obtained by the Fischer ester distillation method (*cf.* 48), by fractional crystallization from phenylalanine-rich protein hydrolysates in the presence of large quantities of sodium chloride (18), and by precipitation with 2,5-dibromobenzenesulfonic acid (301).

8. Proline

Proline can be isolated from protein hydrolysates, especially gelatin and zein, because, of all the monoamino acids, it alone is soluble in absolute alcohol (*cf.* 48). Other methods which have been used are based on the fact that proline copper is soluble in ethanol (132) and methanol (182, 184, 312), and that proline is precipitated with Reinecke acid (174) or rhodanilic acid (tetrathiocyanatodianilidochromiato acid, $[Cr(CNS)_4(C_6H_5NH_2)_2]H$) (30, 299).

9. Serine

This substance can only be isolated with ease from silk fibroin by precipitation with *p*-hydroxyazobenzene-*p*′-sulfonate after previous removal of tyrosine, glycine, and alanine (301).

I. GENERAL ISOLATION METHODS

Besides the often-described Fischer ester method, Dakin's butyl alcohol extraction procedure and fractional crystallization (*cf.* 48), high-vacuum distillation of butyl esters (148, 149), differential adsorption on charcoal (48, 71), fractional extraction with organic acids (250) and specific organic reagents (92, 232, 233, 301, etc.) have been used for separating α-amino acids.

Free amino acids can be recovered from their sodium salts by treating with carbon dioxide under pressure in the presence of an equimolar amount of ammonium bicarbonate and removing the precipitate of sodium bicarbonate (143) or from their hydrochlorides by adding an excess of an amine such as aniline, pyridine, γ-methylbutylamine, etc., or pinene, and extracting the amine hydrochloride or bornyl chloride (16) with chloroform or ethanol (29, 311).

III. SYNTHESIS

Many methods for synthesizing α-amino acids have been proposed since 1850, when A. Strecker (302) prepared alanine by treating acetaldehyde with hydrogen cyanide and ammonia, followed by hydrolysis of the resulting aminocyanohydrin.

It is hoped to present the general methods of synthesis of amino acids with a brief historical picture, although neither the experimental details nor the complete literature will be given. The reader is referred to the excellent reviews by Dunn (94, 95), Clarke (74), and Carter (68) for other details of this subject.

A. AMINATION OF α-HALOGEN ACIDS

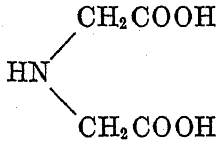
1. From monocarboxylic acids

(a) Preparation of glycine

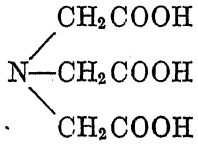
In 1858, Cahours (66) prepared glycine by treating chloroacetic acid with 2 moles of ammonia. This preparation was confirmed by Perkin and Duppa (245) by the synthesis of glycine starting with acetic acid.

$$CH_3COOH + Br_2 \xrightarrow{150°C.} CH_2BrCOOH + HBr \xrightarrow{2NH_3} \underset{\text{Glycine}}{CH_2NH_2COOH} + NH_4Br$$

Since that time, amination of chloroacetic acid has been one of the best methods for preparing glycine. The formation of by-products such as



and



is minimized by the use of a large excess of ammonium hydroxide, sixty times theory (13, 53, 70, 240, 258, 310), liquid ammonia (283), ammonia containing ammonium carbonate (72), or ammonia plus ammonium chloride (109). Glycine is separated from ammonium chloride formed in the reaction by isolation through the copper salt (93) or by precipitation with methanol (13, 53, 240, 310). Krause (193) prepared glycine by amination of the copper salt of chloroacetic acid, eliminating both by-products and ammonium chloride simultaneously.

(b) Synthesis of alanine, valine, leucine, norleucine, etc.

Synthesis of amino acids other than glycine by direct amination of the α-halogen acid is limited by the availability of the halogen acid and by the increasing resistance of higher and branched α-halogen acids to amination. However, alanine has been synthesized in 70–80 per cent yield from bromopropionic acid and in lower yields from chloropropionic acid (157, 309).

$$CH_3CH_2COOH + \text{red P} + Br_2 \rightarrow CH_3CH_2COBr + H_3PO_4$$

$$+ HBr \xrightarrow[40\text{–}50°C.]{Br_2} CH_3CHBrCOBr \xrightarrow{H_2O} CH_3CHBrCOOH$$

$$\xrightarrow{NH_4OH} \underset{\text{Alanine}}{CH_3CHNH_2COOH}$$

Benzoyl-*dl*-alanine is readily resolved by forming the strychnine salt (242). Norleucine has been made from *n*-caproic acid (1, 73, 225) as follows:

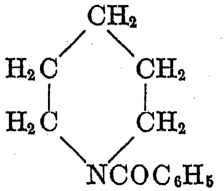
$$CH_3(CH_2)_4COOH \xrightarrow[PCl_3 \text{ or red P}]{Br_2} CH_3(CH_2)_3CHBrCOOH \xrightarrow{NH_4OH}$$

$$\underset{\text{Norleucine}}{CH_3(CH_2)_3CHNH_2COOH}$$

Leucine (222) and valine (220) have also been successfully made by this general reaction (*cf.* 72, 283). The large-scale synthesis of the higher fatty acids from hydrocarbons (156; and other papers by Hass) may permit a much further application of the Cahours–Perkin procedure. Phenylalanine (279) and aspartic acid (177, 326) have also been prepared by this general method.

(c) Synthesis of lysine, proline, and other amino acids

v. Braun prepared lysine from benzoylpiperidine by the following reactions (60):

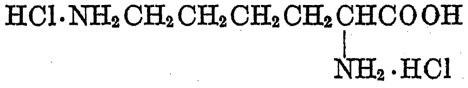


$$\xrightarrow{PCl_5} C_6H_5CONH(CH_2)_5Cl \xrightarrow{KCN}$$

$$C_6H_5CONH(CH_2)_5CN \xrightarrow{KOH} C_6H_5CONH(CH_2)_5COOH \xrightarrow[\text{red P}]{Br_2}$$

$$C_6H_5CONH(CH_2)_4CHBrCOOH \xrightarrow{NH_3}$$

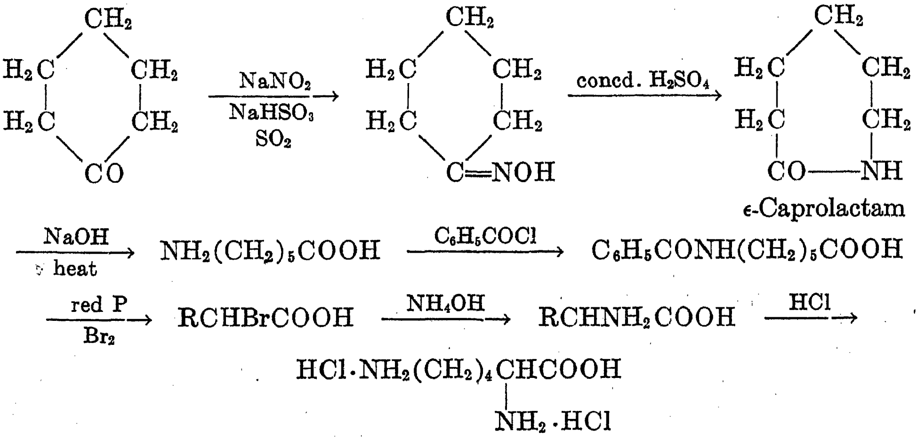
$$C_6H_5CONH(CH_2)_4CHNH_2COOH \xrightarrow{\text{concd. HCl}}$$



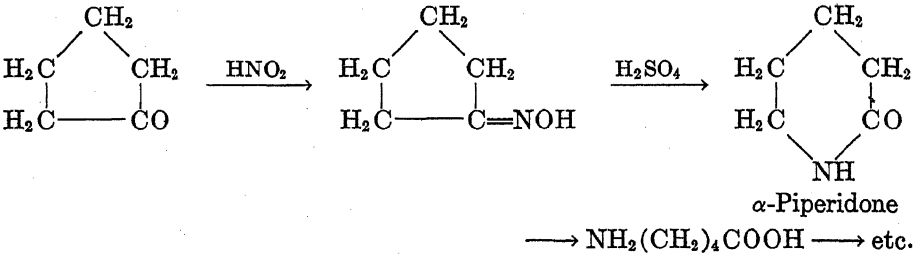
$$HCl\cdot NH_2CH_2CH_2CH_2CH_2\underset{\displaystyle NH_2\cdot HCl}{\underset{|}{C}}HCOOH$$

dl-Lysine dihydrochloride

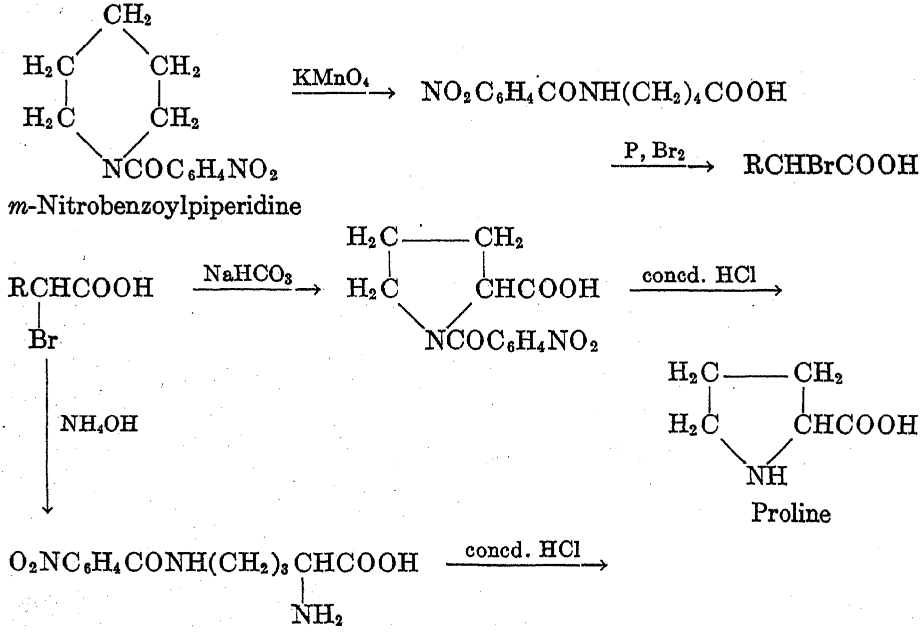
Eck and Marvel (101) used a modification of the v. Braun (60) method for the synthesis of lysine, starting with cyclohexanone and making use of the Beckmann–Wallach rearrangement.



Schniepp and Marvel (273) employed a somewhat similar method for preparing ornithine and arginine, starting with cyclopentanone through the oxime.



Fischer and Zemplén (130) also used a modification of v. Braun's method to prepare ornithine and proline.

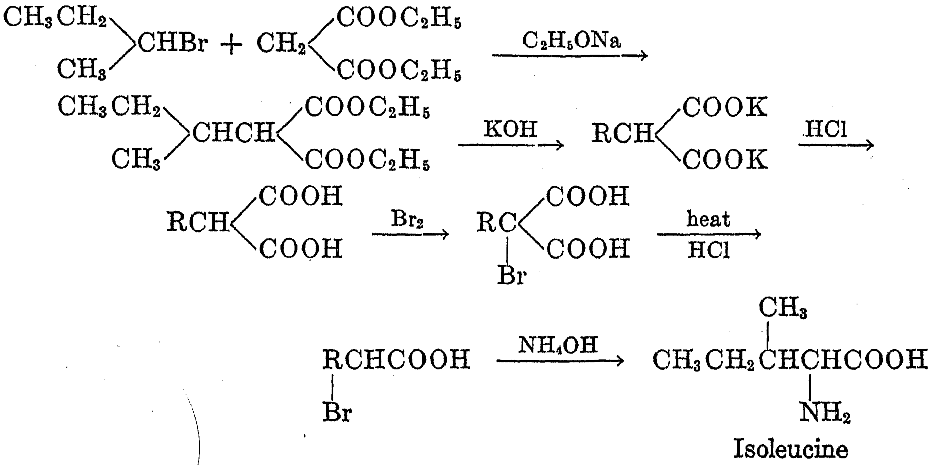


2. From substituted malonic acids

Direct halogenation of monocarboxylic fatty acids is often difficult or impracticable, while the corresponding malonic (1,1-dicarboxylic) acids halogenate readily.

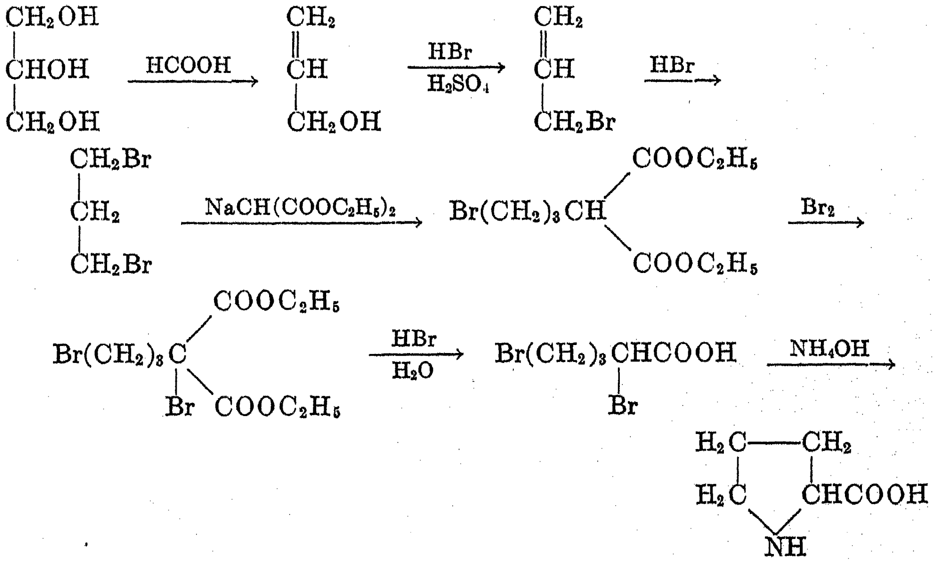
(a) Preparation of valine, isoleucine, norleucine, and phenylalanine

Marvel and associates have developed this procedure for the practical preparation of the above-mentioned amino acids (4, 221, 223, 226). Thus isoleucine (221) is prepared as follows:



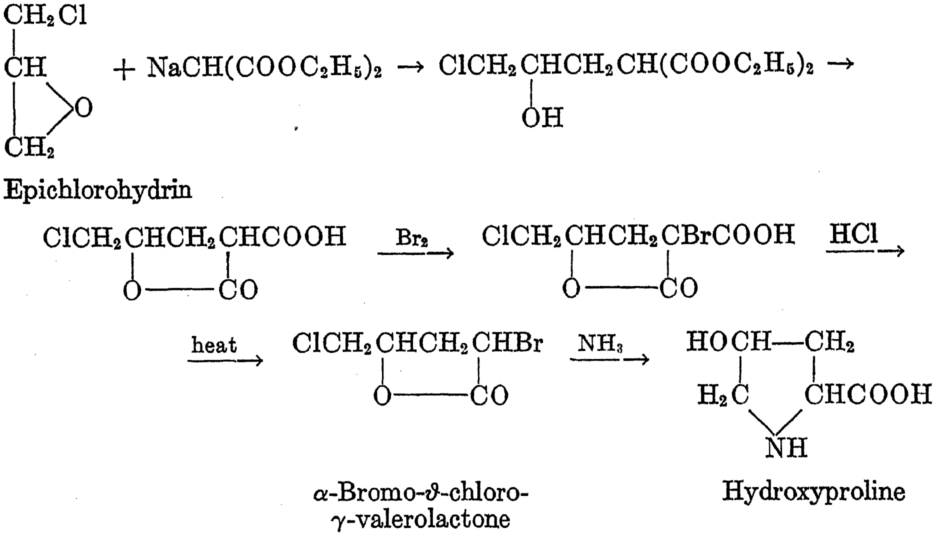
(b) Preparation of proline and hydroxyproline

Willstätter's (136, 336, 337) synthesis of proline from trimethylene bromide and sodiomalonic ester antedates the above use of malonic acid. Proline was synthesized as follows:



γ-Chlorobutyronitrile (158) can also be used to synthesize proline.

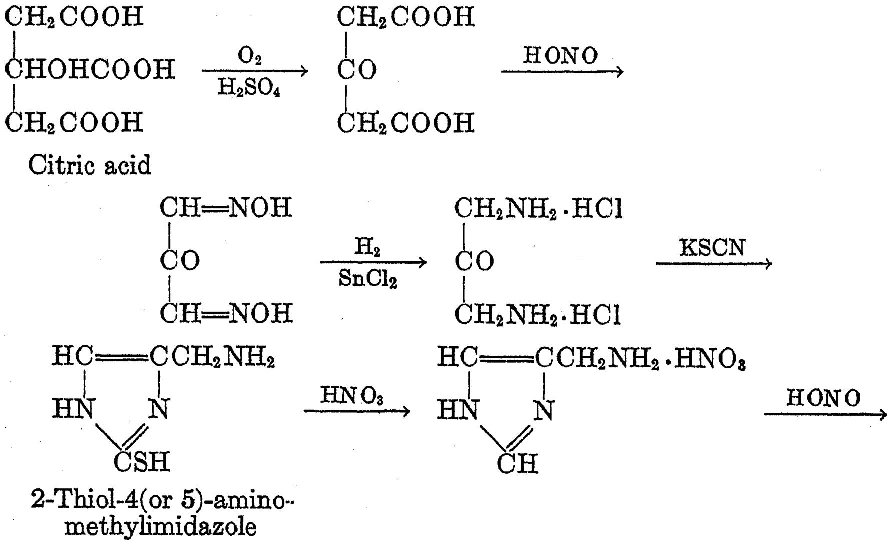
Leuchs and Brewster (200, 201, 202) prepared hydroxyproline from Traube's (316) δ-chloro-γ-valerolactone-α-carboxylic acid as follows:

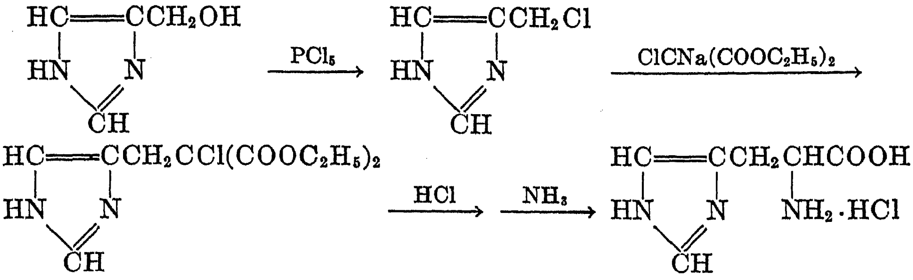


Traube (315) later made some modifications of the Leuchs–Brewster synthesis.

(c) Synthesis of histidine

The brilliant synthesis of histidine by Pyman (253) in 1911 is of theoretical rather than practical interest, although with improved intermediary compounds certain features may prove of practical importance.





Histidine hydrochloride

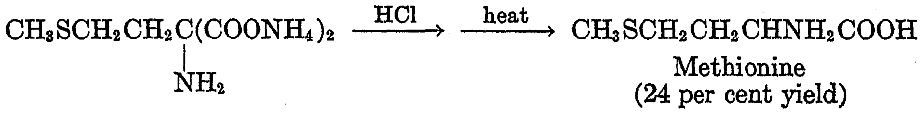
(d) Preparation of methionine and homocystine

Windus and Marvel (338) prepared methionine by the following series of reactions:

$$CH_3SH + ClCH_2CH_2OH \xrightarrow{C_2H_5ONa} CH_3SCH_2CH_2OH \xrightarrow{SOCl_2}$$

$$CH_3SCH_2CH_2Cl \xrightarrow{NaCH(COOC_2H_5)_2} CH_3SCH_2CH_2CH(COOC_2H_5)_2 \longrightarrow$$

$$CH_3SCH_2CH_2CH(COOH)_2 \xrightarrow{Br_2} CH_3SCH_2CH_2CBr(COOH)_2 \xrightarrow{NH_4OH}$$



$$\underset{\substack{|\\NH_2}}{CH_3SCH_2CH_2C(COONH_4)_2} \xrightarrow{HCl} \xrightarrow{heat} CH_3SCH_2CH_2CHNH_2COOH$$

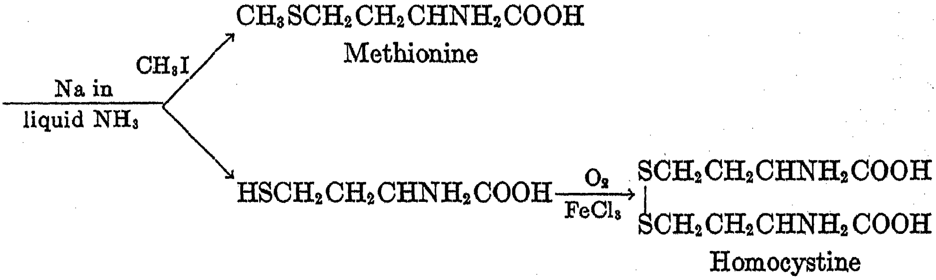
Methionine
(24 per cent yield)

Patterson and du Vigneaud (244) condensed sodium benzyl mercaptan with ethylene chloride and prepared benzylhomocysteine essentially according to the method of Windus and Marvel (338); the benzyl group was then replaced by methyl, utilizing sodium in liquid ammonia for both debenzylation and methylation.

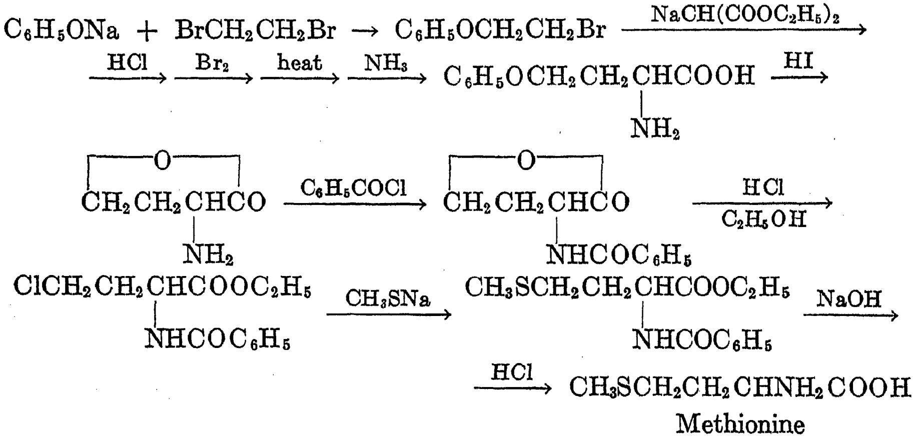
$$C_6H_5CH_2SNa \xrightarrow{ClCH_2CH_2Cl} C_6H_5CH_2SCH_2CH_2Cl \xrightarrow{NaCH(COOC_2H_5)_2}$$

$$C_6H_5CH_2SCH_2CH_2CH(COOC_2H_5)_2 \xrightarrow{KOH} \xrightarrow{Br_2} \xrightarrow{NH_3} \xrightarrow{HCl} \xrightarrow{heat}$$

$$C_6H_5CH_2SCH_2CH_2CHNH_2COOH \longrightarrow$$



Hill and Robson (162) prepared methionine[1] *via* α-amino-γ-butyrolactone, the intermediate steps being essentially according to the following reactions (244, 338):



$$C_6H_5ONa + BrCH_2CH_2Br \rightarrow C_6H_5OCH_2CH_2Br \xrightarrow{NaCH(COOC_2H_5)_2}$$

$$\xrightarrow{HCl} \xrightarrow{Br_2} \xrightarrow{heat} \xrightarrow{NH_3} \underset{\displaystyle NH_2}{C_6H_5OCH_2CH_2CHCOOH} \xrightarrow{HI}$$

$$\underset{\displaystyle NH_2}{\overbrace{CH_2CH_2CHCO}^{O}} \xrightarrow{C_6H_5COCl} \underset{\displaystyle NHCOC_6H_5}{\overbrace{CH_2CH_2CHCO}^{O}} \xrightarrow[C_2H_5OH]{HCl}$$

$$\underset{\displaystyle NHCOC_6H_5}{ClCH_2CH_2CHCOOC_2H_5} \xrightarrow{CH_3SNa} \underset{\displaystyle NHCOC_6H_5}{CH_3SCH_2CH_2CHCOOC_2H_5} \xrightarrow{NaOH}$$

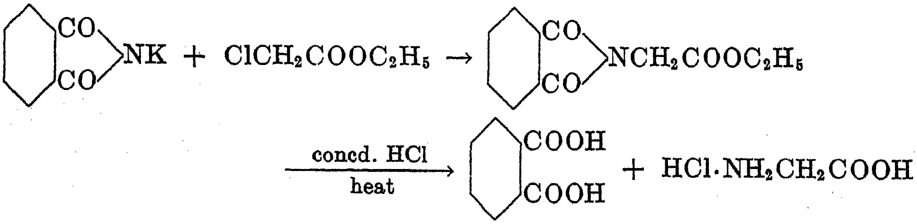
$$\xrightarrow{HCl} CH_3SCH_2CH_2CHNH_2COOH$$

Methionine

3. From potassium (sodium) phthalimide

(a) Gabriel synthesis

In 1888 Goedeckemeyer (145), working in Gabriel's laboratory, synthesized glycine, using potassium phthalimide and chloroacetic ester.



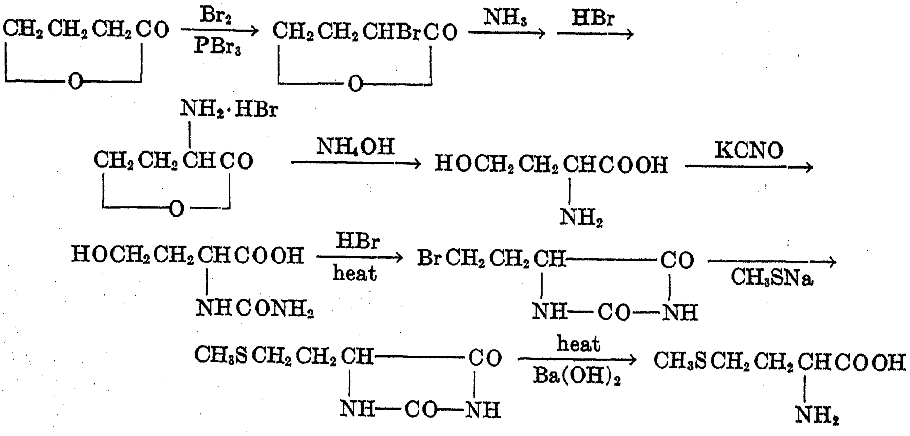
$$C_6H_4(CO)_2NK + ClCH_2COOC_2H_5 \rightarrow C_6H_4(CO)_2NCH_2COOC_2H_5$$

$$\xrightarrow[heat]{concd.\ HCl} C_6H_4(COOH)_2 + HCl{\cdot}NH_2CH_2COOH$$

Glycine hydrochloride

---

[1] Livak *et al.* (209a) have synthesized *dl*-methionine by the following reactions:



$$\underbrace{CH_2CH_2CH_2CO}_{O} \xrightarrow[PBr_3]{Br_2} \underbrace{CH_2CH_2CHBrCO}_{O} \xrightarrow{NH_3} \xrightarrow{HBr}$$

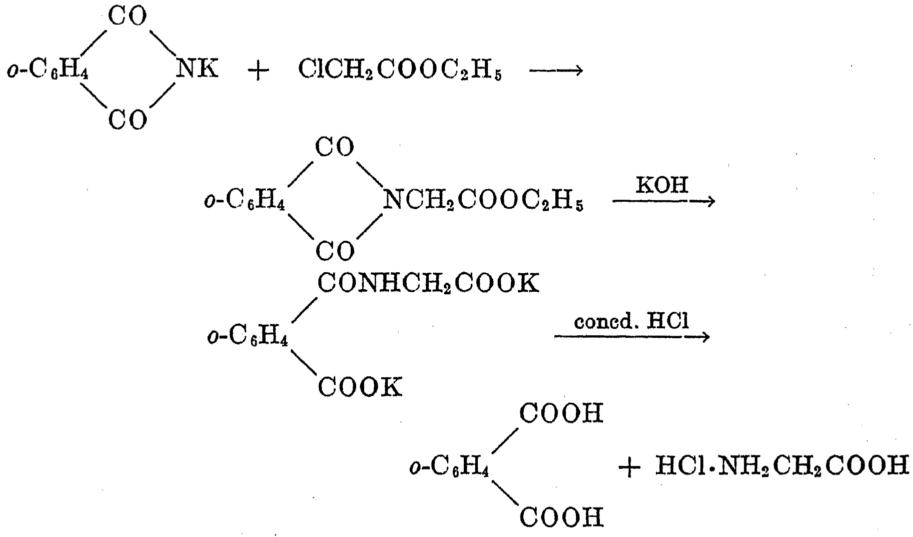
$$\underbrace{CH_2CH_2\overset{\displaystyle NH_2{\cdot}HBr}{CH}CO}_{O} \xrightarrow{NH_4OH} \underset{\displaystyle NH_2}{HOCH_2CH_2CHCOOH} \xrightarrow{KCNO}$$

$$\underset{\displaystyle NHCONH_2}{HOCH_2CH_2CHCOOH} \xrightarrow[heat]{HBr} \underset{\displaystyle NH—CO—NH}{BrCH_2CH_2CH—CO} \xrightarrow[CH_3SNa]{}$$

$$\underset{\displaystyle NH—CO—NH}{CH_3SCH_2CH_2CH—CO} \xrightarrow[Ba(OH)_2]{heat} \underset{\displaystyle NH_2}{CH_3SCH_2CH_2CHCOOH}$$

dl-Methionine

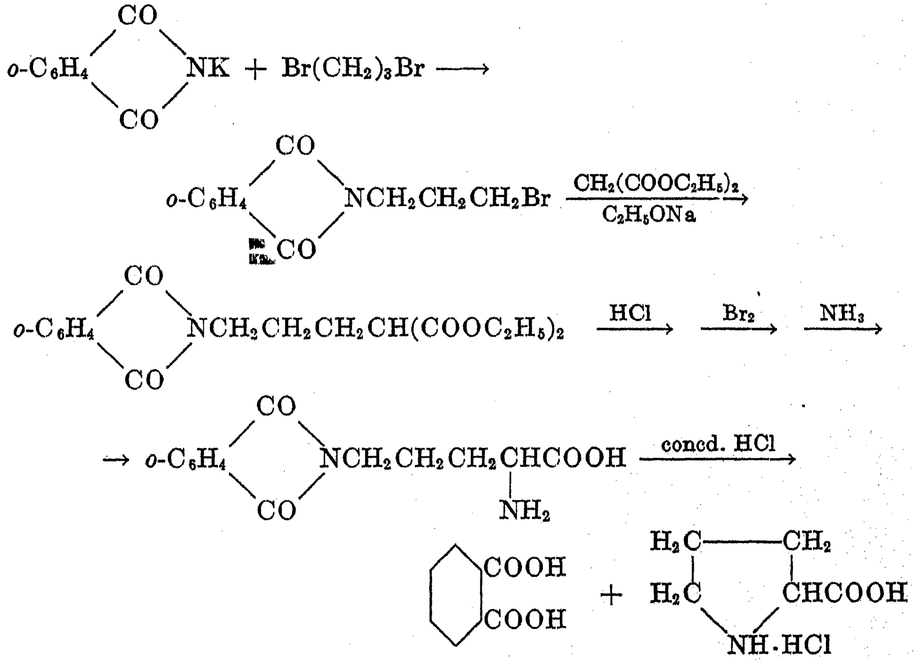
The conditions were somewhat changed by Gabriel and Kroseberg (138; *cf.* 274) the following year. This reaction was to become the basis of the Sørensen phthalimidomalonic ester method and similar methods which will be described later in this paper.



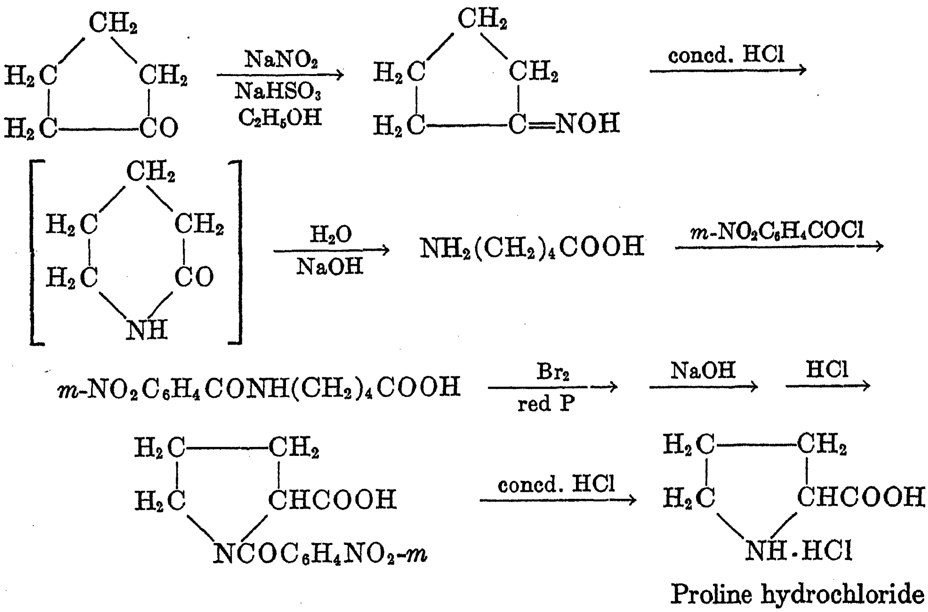
Glycine hydrochloride

4. Miscellaneous methods

Fischer (125) utilized both potassium phthalimide and malonic ester in his synthesis of proline.



Dunn (95) used a combination of Marvel's (101, 273) cyclopentanone method and Fischer's method (130) to prepare proline.



Schroeter (276, 277) used double decomposition of *p*-toluenesulfonamide and α-halogen acids for the preparation of amino acids. The toluenesulfonamide is easily recovered.

$$p\text{-}CH_3C_6H_4SO_2NH_2 + ClCH_2COOH \xrightarrow{NaOH}$$

$$p\text{-}CH_3C_6H_4SO_2NHCH_2COOH \xrightarrow[ClCH_2COCl]{CH_3COCl\text{ or}}$$

$$p\text{-}CH_3C_6H_4SO_2Cl + \underset{\text{Glycine}}{NH_2CH_2COOH}$$

Alanine is similarly prepared from α-bromopropionic acid.

Gaudry (140, 140a) has recently prepared phenylalanine and tyrosine from benzenediazonium and *p*-methoxybenzenediazonium chlorides and acrylonitrile, all easily available compounds. This reaction appears promising.

$$C_6H_5N_2Cl + CH_2{=}CHCN \rightarrow C_6H_5CH_2CHClCN \xrightarrow{H_2SO_4}$$

$$C_6H_5CH_2CHClCONH_2 \xrightarrow[HCl]{heat} C_6H_5CH_2CHClCOOH \xrightarrow[(NH_4)_2CO_3]{NH_4OH}$$

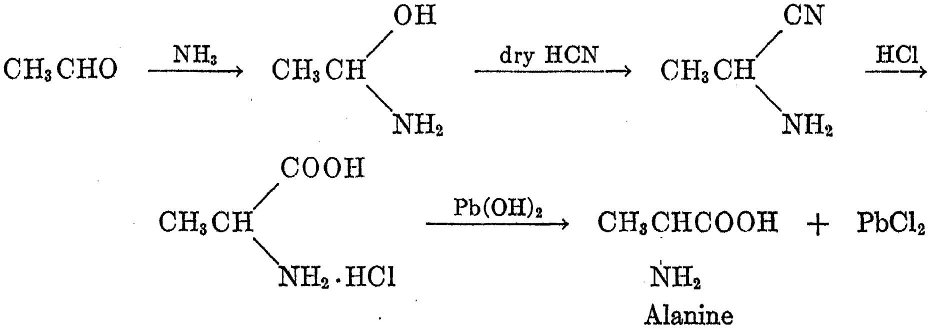
$$C_6H_5CH_2CHNH_2COONH_4$$

B. HYDROLYSIS OF AMINOCYANOHYDRINS (STRECKER SYNTHESIS)

In contrast to the many modifications which the synthesis of amino acids by the amination of α-halogen fatty acids has undergone, Strecker's aminocyanohydrin synthesis has been changed in minor detail only during the past 100 years.

1. Alanine

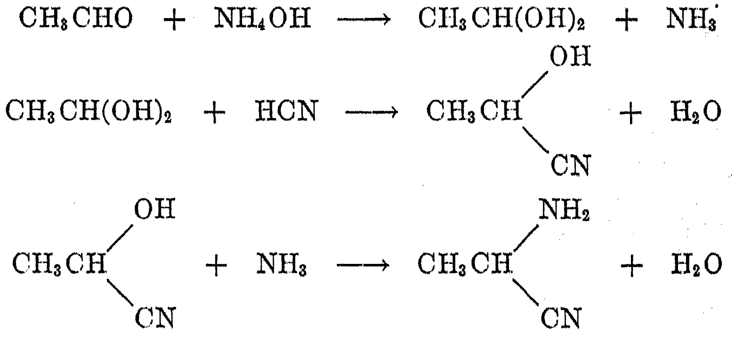
In 1850, A. Strecker (320) treated the addition product of acetaldehyde and ammonia with anhydrous hydrogen cyanide. He hydrolyzed the resulting aminocyanohydrin with hydrochloric acid and, after removing the bound hydrochloric acid with lead hydroxide, recognized that the resulting α-amino acid was the next higher homologue of glycine. Although the structure of glycine had been elucidated a few years previously, this acid was not to be synthesized until eight years later by Cahours (66). Thus the first synthetically prepared α-amino acid was named *alanine* after its mother substance, aldehyde.



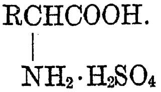
The order of adding ammonia and hydrogen cyanide may be reversed.

Lubavin (210) treated acetaldehyde with ammonium cyanide to obtain the aminocyanohydrin in one step, while Barker (21) and Kendall (178) used a mixture of ammonium chloride and sodium cyanide to achieve the same result. Liquid hydrogen cyanide and concentrated ammonium hydroxide (76) or ammonia gas (167) or liquid ammonia (229) have also been used.

The mechanism of the Strecker synthesis has been studied by Sannie (263), who formulates the reaction as follows:

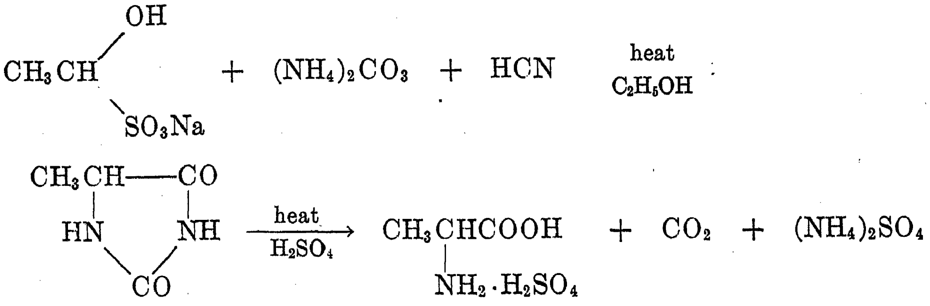


Other mechanisms and by-products are also given. Brodkorb (62) saponifies the aminonitriles with just enough sulfuric acid to form ammonium bisulfate and



$$\underset{\displaystyle NH_2 \cdot H_2SO_4}{RCHCOOH}.$$

Bucherer (63, 64) treated the aldehyde or the aldehyde bisulfite addition product with ammonium carbonate and hydrogen cyanide in hot ethanol and isolated the hydantoin, which was then hydrolyzed to the amino acid.



Methylhydantoin

2. Glycine

Jay and Curtius (169) observed that potassium cyanide and ammonium chloride reacted with 2 moles of formaldehyde rather than 1 mole, as expected from Strecker's experience with alanine. This product, methyleneaminoacetonitrile, was, however, readily hydrolyzed to glycine or glycine ester hydrochloride.

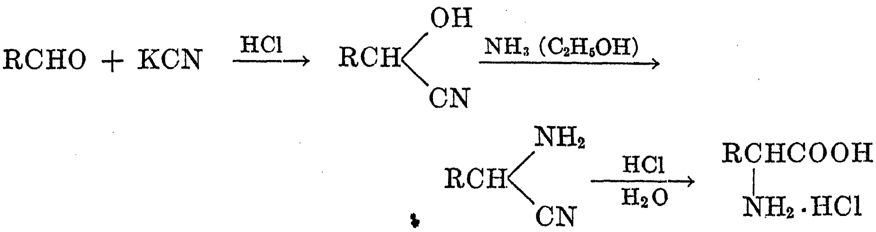
$$2HCHO + KCN + NH_4Cl \xrightarrow{CH_3COOH}$$

$$CH_2{=}NCH_2CN \xrightarrow[\substack{\text{or } Ba(OH)_2 \\ \text{heat}}]{HCl + C_2H_5OH} \underset{\text{Glycine}}{CH_2NH_2COOH}$$

This reaction has been used to prepare glycine or glycine ester hydrochloride in relatively large quantities by Cocking (77), Ling (208), Anslow *et al.* (13), and others.

3. Leucine, valine, isoleucine, and phenylalanine

Fourteen years after the synthesis of alanine by A. Strecker, his brother Hermann Strecker (303) prepared leucine hydrochloride from valeraldehyde by the same method. Lipp (209) synthesized valine from isobutyraldehyde by Strecker's original procedure (*cf.* 183), while Ehrlich (103) and Tiemann (307, 308) prepared isoleucine and phenylalanine, respectively, from the corresponding aldehydes as follows:

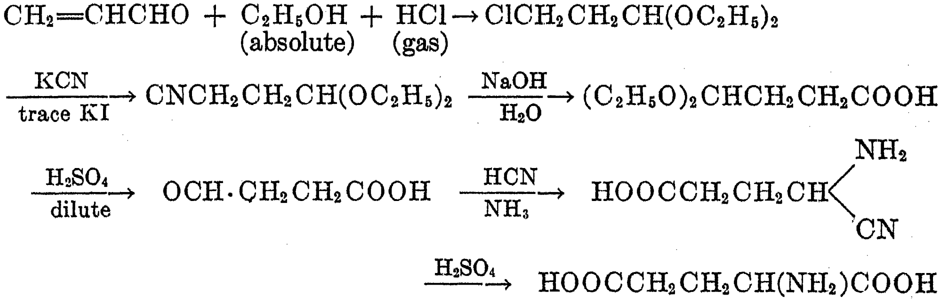


$$\mathrm{RCHO} + \mathrm{KCN} \xrightarrow{\mathrm{HCl}} \mathrm{RCH}{<}\begin{matrix}\mathrm{OH}\\ \mathrm{CN}\end{matrix} \xrightarrow{\mathrm{NH_3\ (C_2H_5OH)}} \mathrm{RCH}{<}\begin{matrix}\mathrm{NH_2}\\ \mathrm{CN}\end{matrix} \xrightarrow[\mathrm{H_2O}]{\mathrm{HCl}} \underset{\mathrm{NH_2\cdot HCl}}{\mathrm{RCHCOOH}}$$

Bucherer and Steiner (64) also synthesized phenylalanine from benzaldehyde, ammonium carbonate, and hydrogen cyanide *via* the hydantoin.

4. *Glutamic acid*

Keimatsu and Sugasawa (176) synthesized glutamic acid from acrolein. This synthesis has academic interest only, as it is far simpler to prepare *dl*-glutamic acid from natural *l*-glutamic acid by racemization.



$$\mathrm{CH_2{=}CHCHO} + \underset{\text{(absolute)}}{\mathrm{C_2H_5OH}} + \underset{\text{(gas)}}{\mathrm{HCl}} \rightarrow \mathrm{ClCH_2CH_2CH(OC_2H_5)_2}$$

$$\xrightarrow[\text{trace KI}]{\mathrm{KCN}} \mathrm{CNCH_2CH_2CH(OC_2H_5)_2} \xrightarrow[\mathrm{H_2O}]{\mathrm{NaOH}} \mathrm{(C_2H_5O)_2CHCH_2CH_2COOH}$$

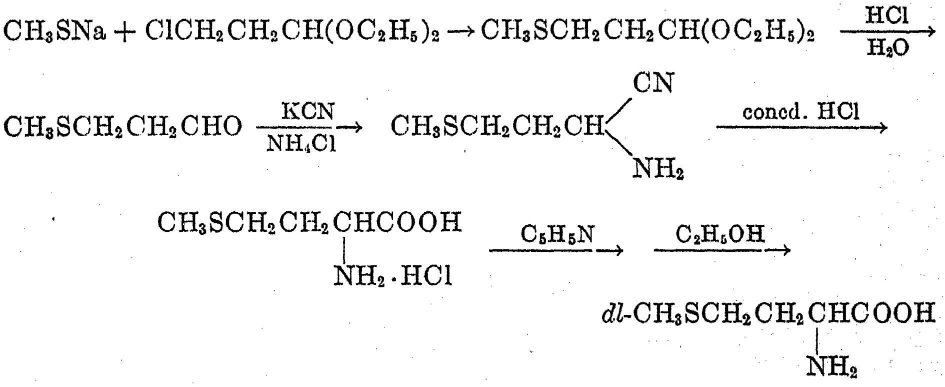
$$\xrightarrow[\text{dilute}]{\mathrm{H_2SO_4}} \mathrm{OCH\cdot CH_2CH_2COOH} \xrightarrow[\mathrm{NH_3}]{\mathrm{HCN}} \mathrm{HOOCCH_2CH_2CH}{<}\begin{matrix}\mathrm{NH_2}\\ \mathrm{CN}\end{matrix}$$

$$\xrightarrow{\mathrm{H_2SO_4}} \mathrm{HOOCCH_2CH_2CH(NH_2)COOH}$$

Glutamic acid

5. *Methionine*

The synthesis of methionine from β-methylthiolpropionaldehyde diethylacetal by Barger and Coyne (19) established the constitution of this amino acid.



$$\mathrm{CH_3SNa} + \mathrm{ClCH_2CH_2CH(OC_2H_5)_2} \rightarrow \mathrm{CH_3SCH_2CH_2CH(OC_2H_5)_2} \xrightarrow[\mathrm{H_2O}]{\mathrm{HCl}}$$

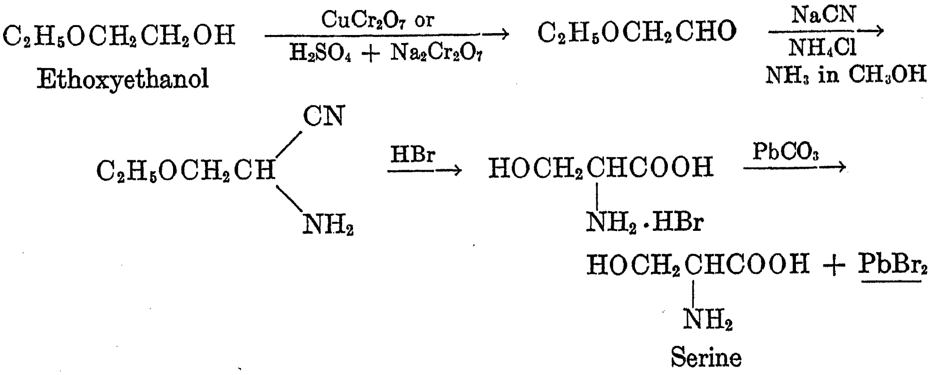
$$\mathrm{CH_3SCH_2CH_2CHO} \xrightarrow[\mathrm{NH_4Cl}]{\mathrm{KCN}} \mathrm{CH_3SCH_2CH_2CH}{<}\begin{matrix}\mathrm{CN}\\ \mathrm{NH_2}\end{matrix} \xrightarrow{\text{concd. HCl}}$$

$$\underset{\mathrm{NH_2\cdot HCl}}{\mathrm{CH_3SCH_2CH_2CHCOOH}} \xrightarrow{\mathrm{C_5H_5N}} \xrightarrow{\mathrm{C_2H_5OH}} dl\text{-}\underset{\mathrm{NH_2}}{\mathrm{CH_3SCH_2CH_2CHCOOH}}$$

Methionine

6. *Serine*

Dunn and Redemann (97, 256) synthesized this amino acid from the readily available ethylene glycol monoethyl ether (Cellosolve).



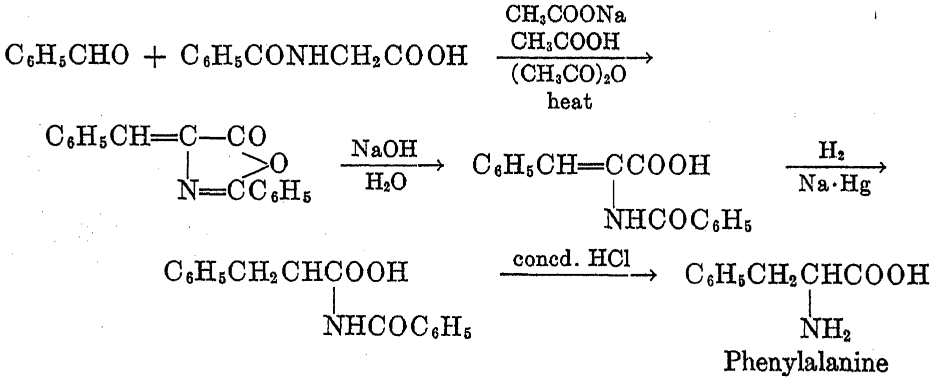
C. CONDENSATION OF AN ALDEHYDE WITH AN ACTIVE METHYLENE GROUP: THE PERKIN REACTION (ERLENMEYER'S SYNTHESIS)

This synthesis is useful for the preparation of the four aromatic amino acids—phenylalanine, tyrosine, tryptophan, and thyroxine, especially the first two.

1. *Phenylalanine and tyrosine*

(a) By condensation with hippuric acid

Although Erlenmeyer and Lipp (114) synthesized tyrosine from *p*-aminophenylalanine by deamination of the *p*-NH_2 group with nitrous acid anhydride as early as 1883, a general method for the ready preparation of phenylalanine and tyrosine was not described until 10 years later (111, 112). This was accomplished by condensing benzaldehyde or *p*-hydroxybenzaldehyde with hippuric acid by heating with a mixture of sodium acetate, acetic acid, and acetic anhydride, to form the lactimide (azlactone). The azlactone ring was opened with alkali, the unsaturated linkage reduced, and the benzoyl group was removed by acid hydrolysis.

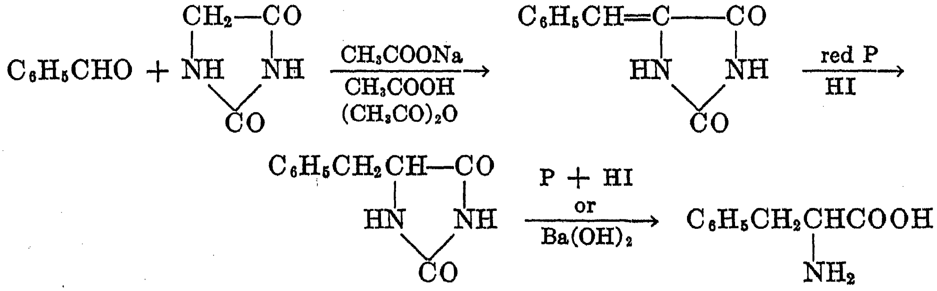


Each step in the original Erlenmeyer hippuric acid synthesis has been modified by various investigators. Thus, Erlenmeyer and Halsey (112) omitted acetic acid, while du Vigneaud *et al.* (325) were unable to obtain efficient condensation (between furfural and hippuric acid) except when fused sodium acetate *alone* was employed.

Various agents have been employed for the reduction of the substituted acrylic acids. Thus, Erlenmeyer (111, 112), Deulofeu (91), and others used sodium amalgam; Harington (155), Lamb (198), and others (163) employed hydrogen iodide, red phosphorus, and glacial acetic acid or acetic anhydride; Johnson used tin and hydrochloric acid (170); Bergmann (31) reduced the double bond catalytically; while Boyd and Robson (51) used ammonium sulfide or sodium hydrosulfide.

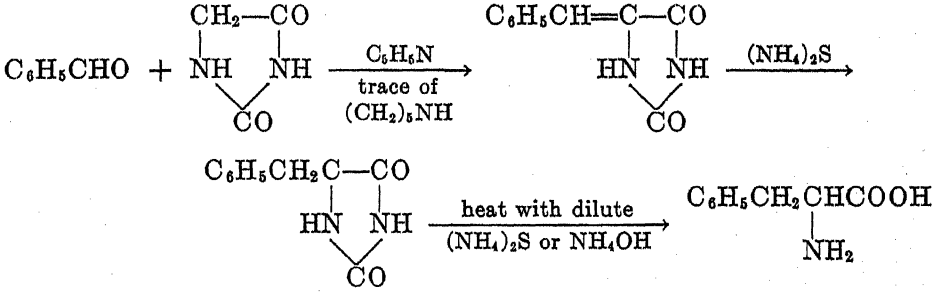
(b) By condensation with hydantoins

Wheeler and Hoffman (333) condensed benzaldehyde or anisaldehyde (*p*-methoxybenzaldehyde) with a preformed cyclic compound containing an active methylene group, i.e., hydantoin.



Phenylalanine

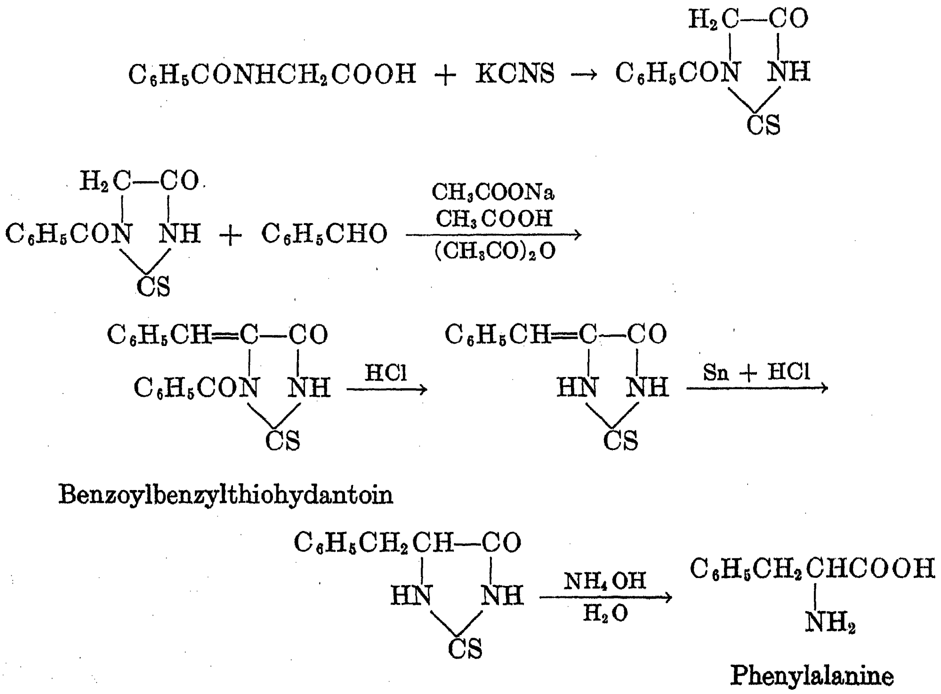
Boyd and Robson (55, 56) replaced Erlenmeyer's acetate condensing agent with an alkaline condensing agent, such as pyridine containing traces of piperidine or diethylamine.



Phenylalanine

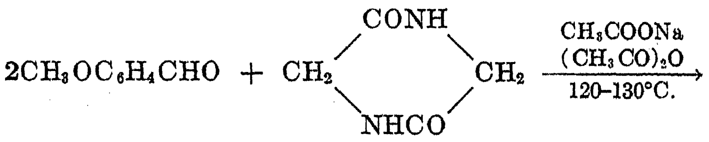
(c) By condensation with thiohydantoin

Johnson and O'Brien (170) prepared a thiohydantoin from hippuric acid and then used the Erlenmeyer–Wheeler method.



(d) By condensation with diketopiperazine (glycine anhydride)

Sasaki (228) used diketopiperazine as the ring structure containing an active methylene group.



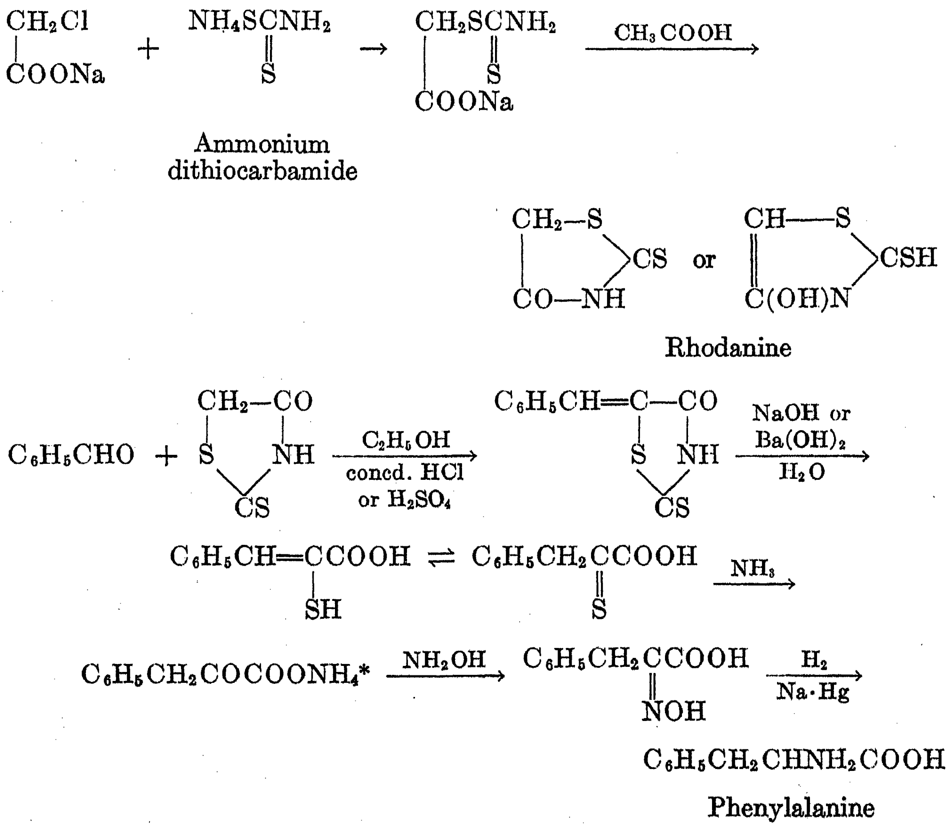
$$\text{3,6-dianisylidene-2,5-diketopiperazine} \xrightarrow[\text{HI}]{\text{red P}}$$

$$p\text{-}HOC_6H_4CH_2CHNH_2COOH + CH_3I$$

Tyrosine

(e) By condensation with rhodanine (2-mercapto-4-oxythiazole)

Gränacher (146, 147) used the well-known reactivity of rhodanine with aromatic aldehydes to prepare amino acids.



Ammonium dithiocarbamide

Rhodanine

Phenylalanine

---

* If NH_2OH is used on sulfhydrylcinnamic acid, the oxime of phenylpyruvic acid is formed directly.

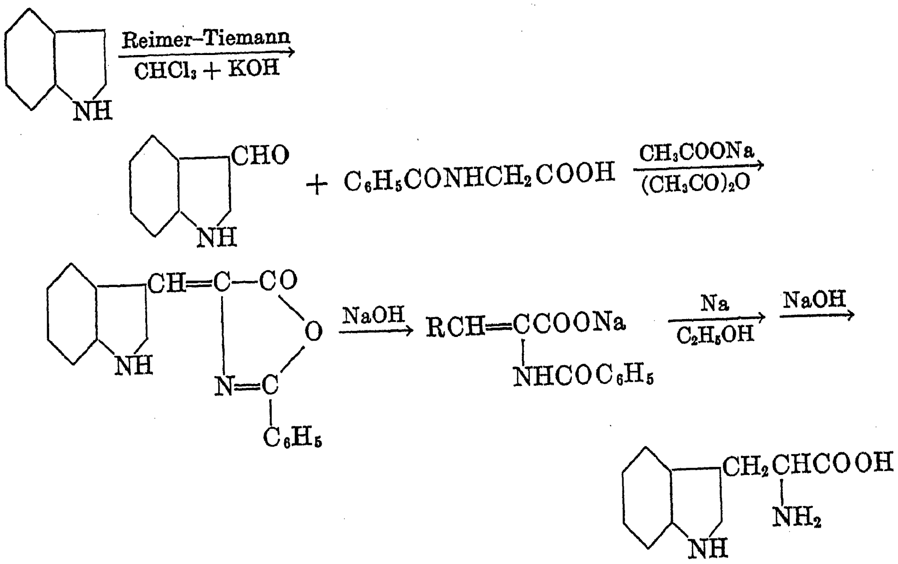
(f) By condensation with acetylglycine

Herbst and Shemin (159) used acetylglycine rather than benzoylglycine (hippuric acid) for the synthesis of phenylalanine.

2. Tryptophan

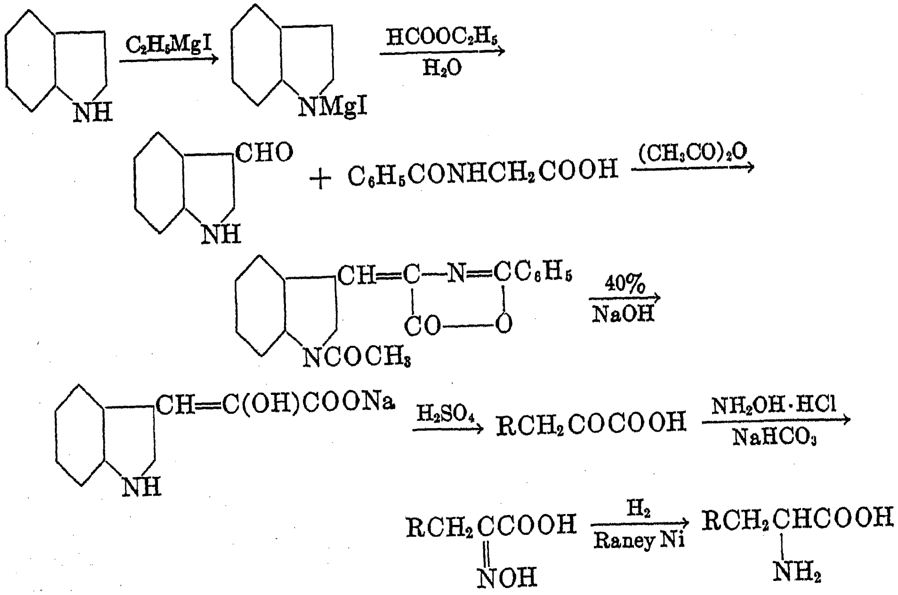
(a) By condensation with hippuric acid

This important amino acid was first synthesized by Ellinger and Flamand (105) according to Erlenmeyer's original method except, of course, avoiding treatment with hot mineral acids.



Tryptophan
(10–17 per cent yield)

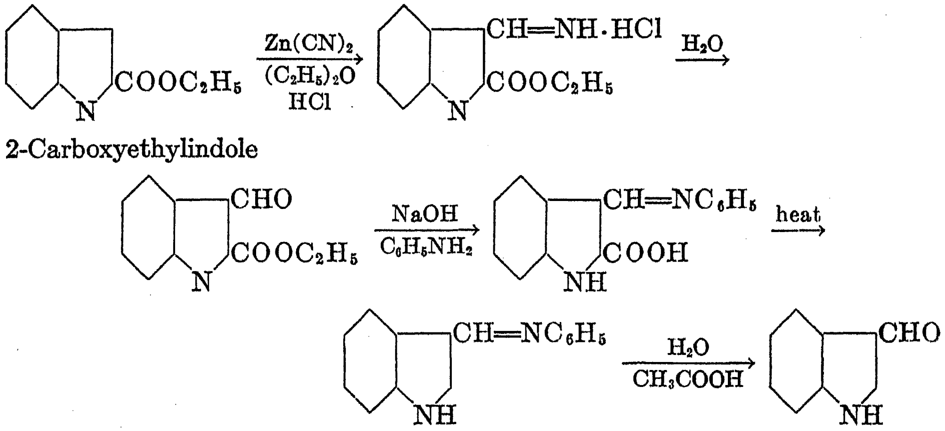
Baugess and Berg (26) prepared indolepyruvic acid by the azlactone method and then made the amino acid by reduction of the oxime. The indolealdehyde was prepared from indole by Majima and Kotake's method (217).



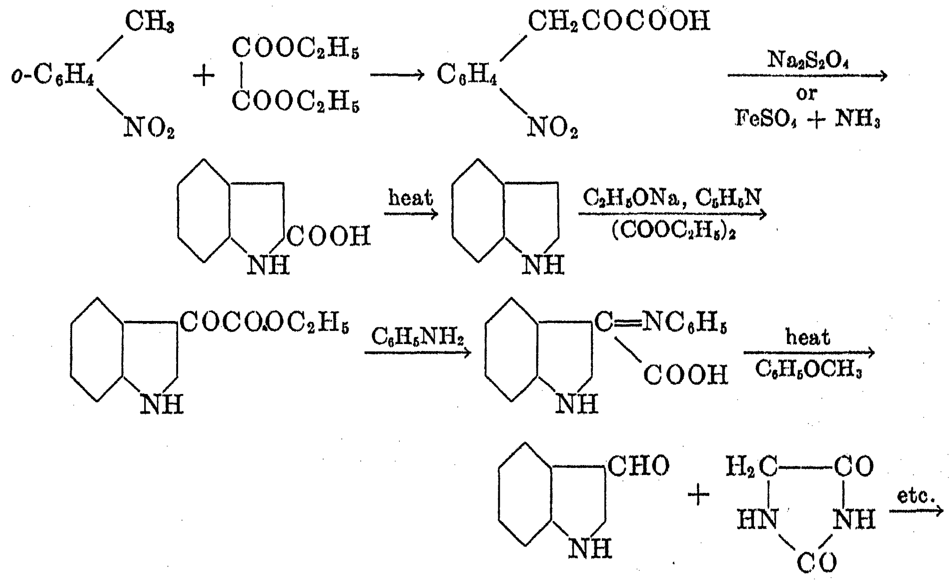
(b) By condensation with hydantoin

Majima and Kotake (217) condensed indolealdehyde, prepared from indolemagnesium iodide (*cf.* above) and ethyl formate, with hydantoin according to Wheeler and Hoffman (333). The β-indolalhydantoin was reduced with sodium amalgam and the hydantylskatole was hydrolyzed with baryta.

Boyd and Robson (58) condensed indole-3-aldehyde with hydantoin, using pyridine–piperidine, and obtained tryptophan from the resulting indolalhydantoin by simultaneous reduction and hydrolysis with ammoniacal ammonium sulfide. Indolealdehyde was prepared either by the Reimer–Tiemann reaction or *via* the Gatterman synthesis (57).



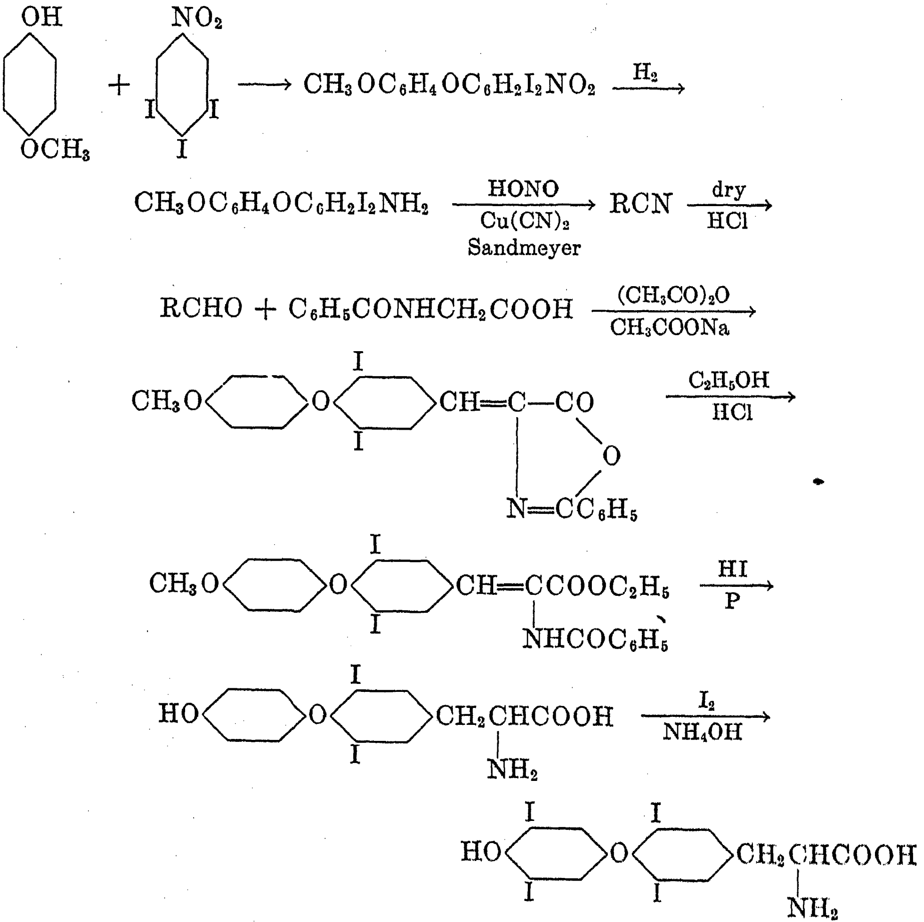
Elks *et al.* (104) prepared indole-3-aldehyde from *o*-nitrotoluene and condensed the former with hydantoin in the usual fashion.



3. Thyroxine

(a) By condensation with benzoylglycine

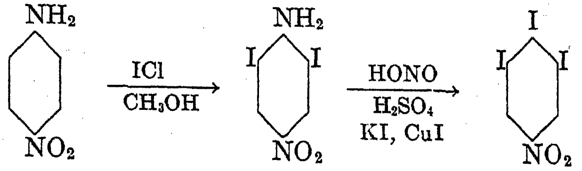
Haringon and Barger (154, 265) prepared thyroxine from the proper aldehyde according to Erlenmeyer's hippuric acid method.



Thyroxine

Thyroxine is [β-(3,5-diiodo-4-(3′,5′-diiodo-4′-hydroxyphenoxy)]phenyl-α-amino-propionic acid.

Triiodonitrobenzene can be prepared from *p*-nitroaniline as follows (265):



4. Histidine

(a) By azlactone synthesis

Pyman (254) used this reaction for his second synthesis of histidine. Glyoxaline formaldehyde was prepared by nitric acid oxidation of the corresponding imidazolylmethyl alcohol. The remainder of the synthesis was essentially according to Erlenmeyer.

5. Lysine

(a) By condensation with malonic acid

Sugasawa (304) has synthesized *dl*-lysine from acrolein in an elaborate procedure involving pyridine–piperidine condensation with malonic acid (*cf.* Section III,B,4 and Section III,C,1,(b)).

$$CH_2{=}CHCHO \xrightarrow[HCl]{C_2H_5OH} ClCH_2CH_2CH(OC_2H_5)_2 \xrightarrow[KCN]{KI}$$

$$CNCH_2CH_2CH(OC_2H_5)_2 \xrightarrow[Na\ +\ C_2H_5OH]{H_2} NH_2CH_2CH_2CH_2CH(OC_2H_5)_2$$

$$\xrightarrow[KOH]{C_6H_5COCl} C_6H_5CONH(CH_2)_3CH(OC_2H_5)_2 \xrightarrow[HCl]{H_2O}$$

$$RCHO + CH_2(COOH)_2 \xrightarrow[\text{trace } C_5H_{11}N]{C_5H_5N} RCH{=}C(COOH)_2 \xrightarrow{H_2} \xrightarrow{\text{heat}}$$

$$RCH_2CH_2COOH \xrightarrow{Br_2} RCH_2CHBrCOOH \xrightarrow{NH_4OH}$$

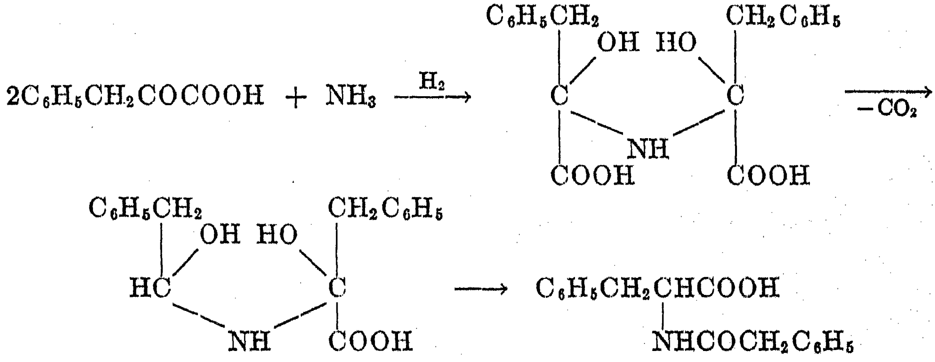
$$RCH_2CHNH_2COOH \xrightarrow[\text{heat}]{HCl} \underset{HCl}{NH_2}CH_2CH_2CH_2CH_2CH\underset{HCl}{NH_2}COOH$$

Lysine dihydrochloride

D. REDUCTION OF α-KETO GROUPS AND THEIR DERIVATIVES

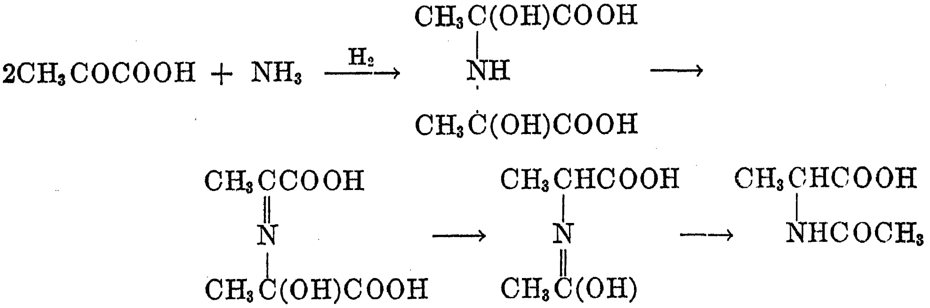
1. Reduction of α-keto groups in the presence of ammonia: alanine, leucine, isoleucine, glutamic acid, etc.

In 1899, Erlenmeyer and Kunlin (113) made the interesting observation that when phenylpyruvic acid was reduced in the presence of ammonia, *N*-phenylacetylphenylalanine was formed.



The phenylacetylphenylalanine could then be hydrolyzed to phenylalanine and phenylacetic acid.

De Jong (173) prepared acetylalanine by reduction of ammonium pyruvate. He formulated the reaction as follows:



$$2CH_3COCOOH + NH_3 \xrightarrow{H_2} \begin{array}{c} CH_3C(OH)COOH \\ | \\ NH \\ | \\ CH_3C(OH)COOH \end{array} \longrightarrow$$

$$\begin{array}{c} CH_3CCOOH \\ \| \\ N \\ | \\ CH_3C(OH)COOH \end{array} \longrightarrow \begin{array}{c} CH_3CHCOOH \\ | \\ N \\ \| \\ CH_3C(OH) \end{array} \longrightarrow \begin{array}{c} CH_3CHCOOH \\ | \\ NHCOCH_3 \end{array}$$

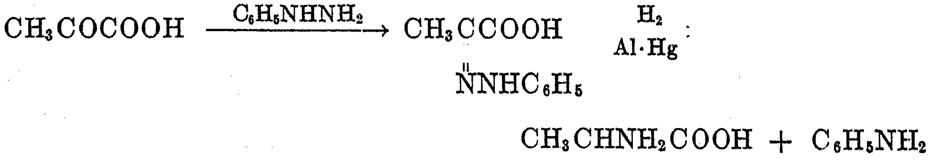
Schoenheimer and Ratner (274), using the active palladium method of Knoop and Oesterlin (186, 187), prepared alanine, phenylalanine, norleucine, tyrosine, aspartic acid, and glutamic acid fron their respective α-keto analogues. When ammonia containing an excess of isotopic nitrogen was used, the resulting amino acids contained an excess of N^{15} in the α-NH_2 group.

$$RCOCOOH + NH_3 \xrightarrow[Pd]{H_2} RCHNH_2COOH + H_2O$$

Other investigators have used palladium or platinum for preparing glycine, alanine, and other amino acids (15, 90, 284). This then appears to be a useful general method for the preparation of α-amino acids provided the corresponding keto acids are available. In fact, Knoop and Oesterlin (186, 187) and others have pointed out that this may be the reaction mechanism for the formation in the body of α-amino acids from α-keto acids.

2. Reduction of phenylhydrazones: alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, etc.

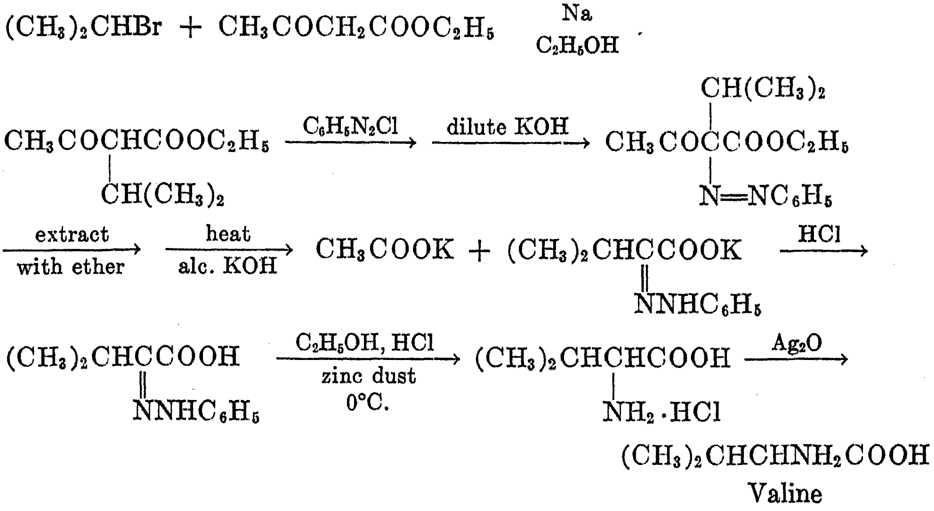
Fischer and Groh (127) obtained a small yield of alanine by reduction of pyruvic acid phenylhydrazone with aluminum amalgam.



$$CH_3COCOOH \xrightarrow{C_6H_5NHNH_2} \begin{array}{c} CH_3CCOOH \\ \| \\ NNHC_6H_5 \end{array} \xrightarrow[Al\cdot Hg]{H_2} CH_3CHNH_2COOH + C_6H_5NH_2$$

Alanine

Feofilaktov and collaborators (116, 117, 118, 121, 122, 123) have modified the Fischer–Groh method into a general procedure for the synthesis of α-amino acids. They have successfully prepared alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, and norleucine from the corresponding substituted acetoacetic esters.



$$(CH_3)_2CHBr + CH_3COCH_2COOC_2H_5 \;\underset{C_2H_5OH}{\overset{Na}{}}$$

$$CH_3CO\underset{CH(CH_3)_2}{CH}COOC_2H_5 \xrightarrow{C_6H_5N_2Cl} \xrightarrow{\text{dilute KOH}} CH_3CO\overset{CH(CH_3)_2}{\underset{N=NC_6H_5}{C}}COOC_2H_5$$

$$\xrightarrow[\text{with ether}]{\text{extract}} \xrightarrow[\text{alc. KOH}]{\text{heat}} CH_3COOK + (CH_3)_2CH\underset{NNHC_6H_5}{\overset{}{C}}COOK \xrightarrow{HCl}$$

$$(CH_3)_2CH\underset{NNHC_6H_5}{C}COOH \xrightarrow[\substack{\text{zinc dust}\\ 0°C.}]{C_2H_5OH,\ HCl} (CH_3)_2CH\underset{NH_2\cdot HCl}{CH}COOH \xrightarrow{Ag_2O}$$

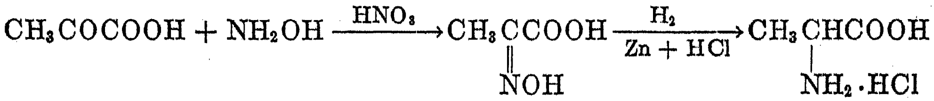
$$\underset{\text{Valine}}{(CH_3)_2CHCHNH_2COOH}$$

Very high yields are reported.

3. Reduction of oximes

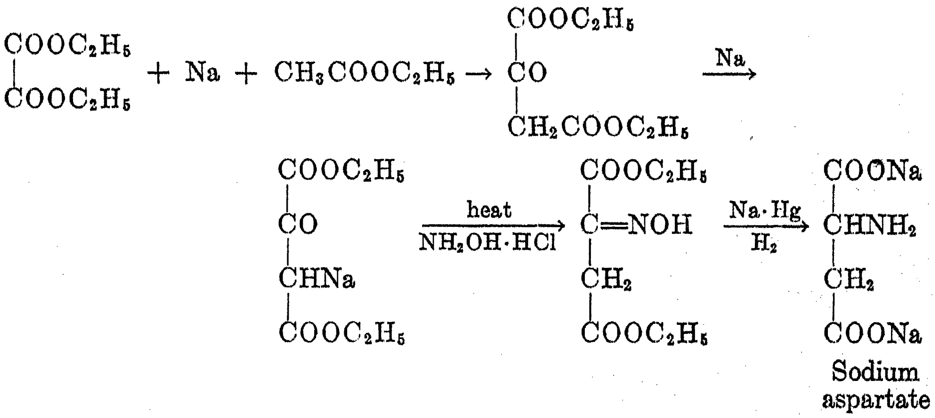
(a) Preparation of oxime with hydroxylamine: aspartic acid, asparagine, glutamic acid, alanine, phenylalanine, tyrosine, etc.

Gutknecht (150) in 1880 prepared alanine by the reduction of pyruvic acid oxime (*cf.* 280).



$$CH_3COCOOH + NH_2OH \xrightarrow{HNO_3} CH_3\underset{NOH}{C}COOH \xrightarrow[Zn + HCl]{H_2} CH_3\underset{NH_2\cdot HCl}{CH}COOH$$

A few years later, Piutti (247) used this observation in his classical synthesis of aspartic acid from oxalacetic ester.

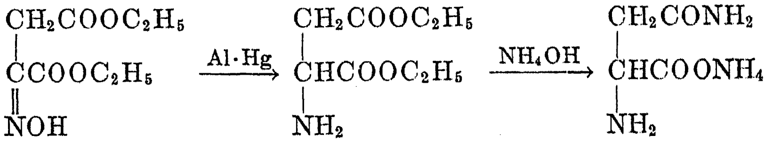


$$\begin{matrix}COOC_2H_5\\ |\\ COOC_2H_5\end{matrix} + Na + CH_3COOC_2H_5 \rightarrow \begin{matrix}COOC_2H_5\\ |\\ CO\\ |\\ CH_2COOC_2H_5\end{matrix} \xrightarrow{Na}$$

$$\begin{matrix}COOC_2H_5\\ |\\ CO\\ |\\ CHNa\\ |\\ COOC_2H_5\end{matrix} \xrightarrow[NH_2OH\cdot HCl]{\text{heat}} \begin{matrix}COOC_2H_5\\ |\\ C{=}NOH\\ |\\ CH_2\\ |\\ COOC_2H_5\end{matrix} \xrightarrow[H_2]{Na\cdot Hg} \begin{matrix}COONa\\ |\\ CHNH_2\\ |\\ CH_2\\ |\\ COONa\\ \text{Sodium}\\ \text{aspartate}\end{matrix}$$

Granacher (146, 147) also used sodium amalgam to reduce ketoximes, Baugess and Berg (26) and Shemin and Herbst (280) used nickel and platinic oxide, respectively, while β-amino acids have been prepared by the electrolytic reduction of substituted β-oximinoacetoacetic esters (14, 259, 267, 332).

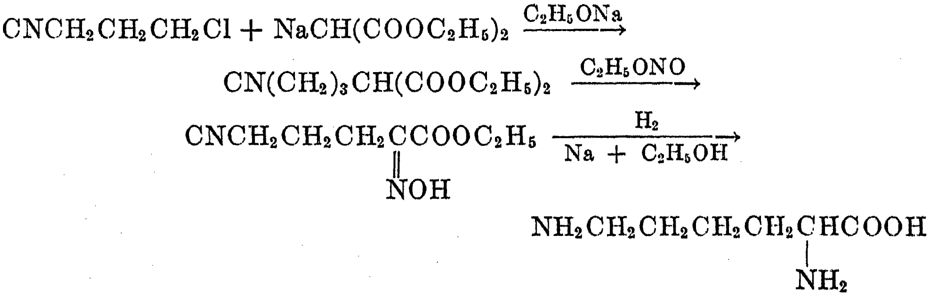
Cocker (75) utilized Piutti's (247) method to prepare asparagine.



$$\begin{array}{l} CH_2COOC_2H_5 \\ | \\ CCOOC_2H_5 \\ \| \\ NOH \end{array} \xrightarrow{Al\cdot Hg} \begin{array}{l} CH_2COOC_2H_5 \\ | \\ CHCOOC_2H_5 \\ | \\ NH_2 \end{array} \xrightarrow{NH_4OH} \begin{array}{l} CH_2CONH_2 \\ | \\ CHCOONH_4 \\ | \\ NH_2 \end{array}$$

(b) Preparation of oxime by action of nitrite on substituted malonic acid or ester: lysine, leucine, phenylalanine

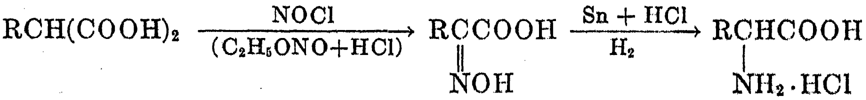
Fischer and Weigert (129) synthesized lysine from γ-chlorobutyronitrile and malonic ester by means of ethyl nitrite.



$$\begin{array}{l} CNCH_2CH_2CH_2Cl + NaCH(COOC_2H_5)_2 \xrightarrow{C_2H_5ONa} \\ \quad CN(CH_2)_3CH(COOC_2H_5)_2 \xrightarrow{C_2H_5ONO} \\ \quad \begin{array}{l} CNCH_2CH_2CH_2CCOOC_2H_5 \\ \qquad\qquad\qquad\quad \| \\ \qquad\qquad\qquad\; NOH \end{array} \xrightarrow[Na\ +\ C_2H_5OH]{H_2} \\ \qquad\qquad \begin{array}{r} NH_2CH_2CH_2CH_2CH_2CHCOOH \\ | \quad\;\; \\ NH_2 \quad \end{array} \end{array}$$

Lysine

Onishchenko (239) modified Fischer's method to prepare leucine and phenylalanine in good yields.

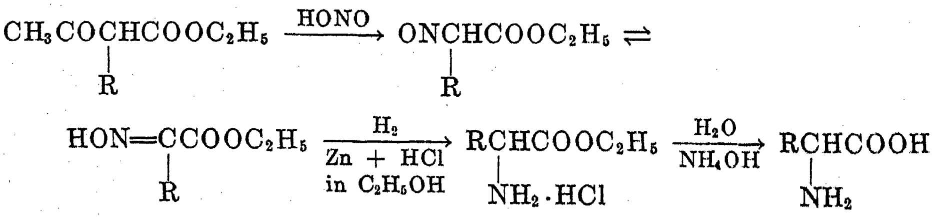


$$RCH(COOH)_2 \xrightarrow[(C_2H_5ONO+HCl)]{NOCl} \begin{array}{l} RCCOOH \\ \| \\ NOH \end{array} \xrightarrow[H_2]{Sn\ +\ HCl} \begin{array}{l} RCHCOOH \\ | \\ NH_2\cdot HCl \end{array}$$

(c) Preparation of oxime by action of nitrite on substituted acetoacetic ester: methionine, threonine, hydroxyproline, aspartic acid, glutamic acid, leucine, isoleucine, etc.

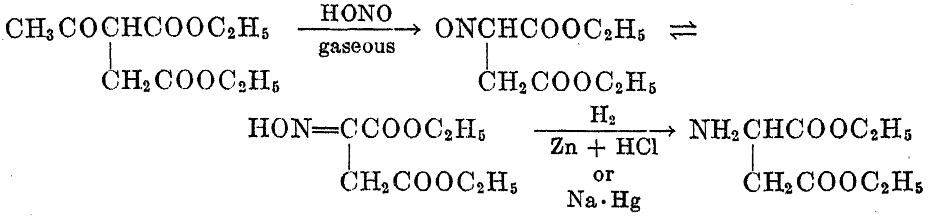
Bouveault and Locquin (54) used acetoacetic ester rather than malonic ester in the preparation of amino acids. Since their publication in 1906, numerous investigators have used this method for the synthesis of many amino acids.

(1) *Alanine*, *valine*, and *isoleucine* by Bouveault and Locquin (54).



$$\begin{array}{l} \begin{array}{l} CH_3COCHCOOC_2H_5 \\ \qquad\;\; | \\ \qquad\;\; R \end{array} \xrightarrow{HONO} \begin{array}{l} ONCHCOOC_2H_5 \\ \quad\;\; | \\ \quad\;\; R \end{array} \rightleftarrows \\ \quad \begin{array}{l} HON{=}CCOOC_2H_5 \\ \qquad\;\; | \\ \qquad\;\; R \end{array} \xrightarrow[\substack{Zn\ +\ HCl \\ in\ C_2H_5OH}]{H_2} \begin{array}{l} RCHCOOC_2H_5 \\ \;\; | \\ NH_2\cdot HCl \end{array} \xrightarrow[NH_4OH]{H_2O} \begin{array}{l} RCHCOOH \\ \;\; | \\ NH_2 \end{array} \end{array}$$

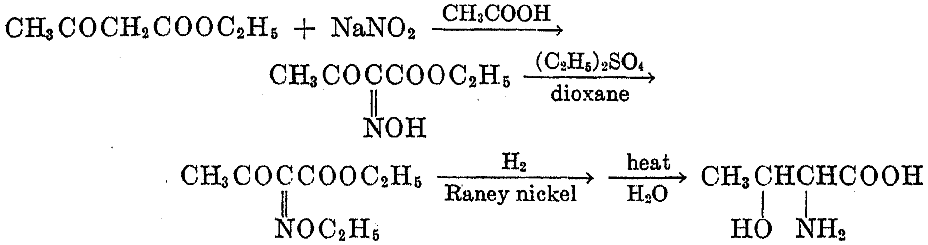
(*2*) *Aspartic acid* by Schmidt and Widmann (271).



$$\underset{\textstyle |\atop CH_2COOC_2H_5}{CH_3COCHCOOC_2H_5} \xrightarrow[\text{gaseous}]{\text{HONO}} \underset{\textstyle |\atop CH_2COOC_2H_5}{ONCHCOOC_2H_5} \rightleftharpoons$$

$$\underset{\textstyle |\atop CH_2COOC_2H_5}{HON{=}CCOOC_2H_5} \xrightarrow[\text{Zn + HCl or Na}\cdot\text{Hg}]{H_2} \underset{\textstyle |\atop CH_2COOC_2H_5}{NH_2CHCOOC_2H_5}$$

(*3*) *Threonine* by Adkins and Reeve (6).



$$CH_3COCH_2COOC_2H_5 + NaNO_2 \xrightarrow{CH_3COOH}$$

$$\underset{\textstyle \|\atop NOH}{CH_3COCCOOC_2H_5} \xrightarrow[\text{dioxane}]{(C_2H_5)_2SO_4}$$

$$\underset{\textstyle \|\atop NOC_2H_5}{CH_3COCCOOC_2H_5} \xrightarrow[\text{Raney nickel}]{H_2} \xrightarrow[H_2O]{\text{heat}} \underset{\textstyle HO\quad NH_2}{CH_3CHCHCOOH}$$

Ethyl *O*-ethyloximino-acetoacetate

Threonine + allothreonine

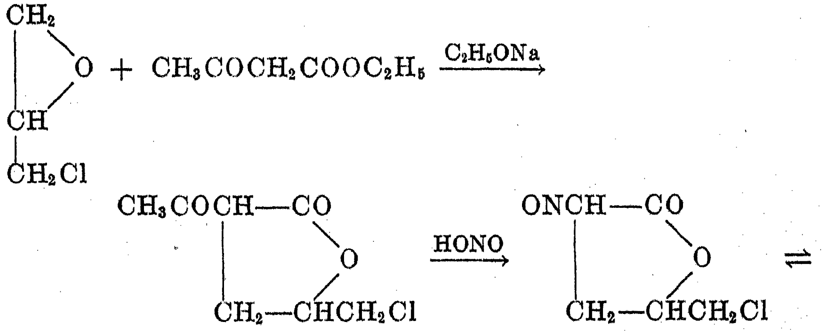
It should be noted that this synthesis does not involve a substituted acetoacetic ester and that the acetyl group is not removed by nitrosation.

(*4*) *Other amino acids:* McIlwain and Richardson (228) used the Bouveault–Locquin method for glutamic acid (good yield) and hydroxyproline (poor yield). They were unable to prepare threonine, as the hydroxyl group was reduced by their platinic oxide catalyst.

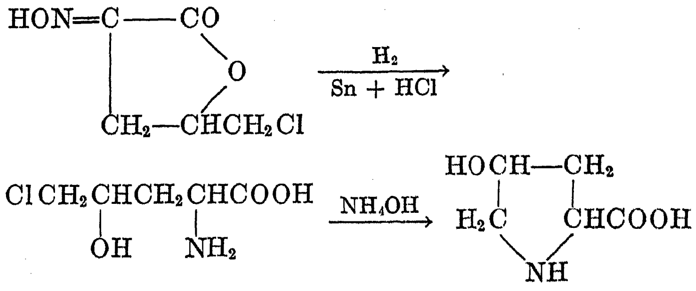
Hamlin and Hartung (151) prepared tyrosine, isoleucine, and a number of other amino acids by treatment of substituted acetoacetic esters with butyl nitrite (*cf.* 236) and sulfuric acid. The α-oximino esters were reduced with hydrogen and palladium. The yields were very good.

(d) Syntheses from α-oximino-γ-butyrolactone

(*1*) *Hydroxyproline:* Feofilaktov (119, 120) used the action of nitrous acid on an acetobutyrolactone to prepare hydroxyproline.

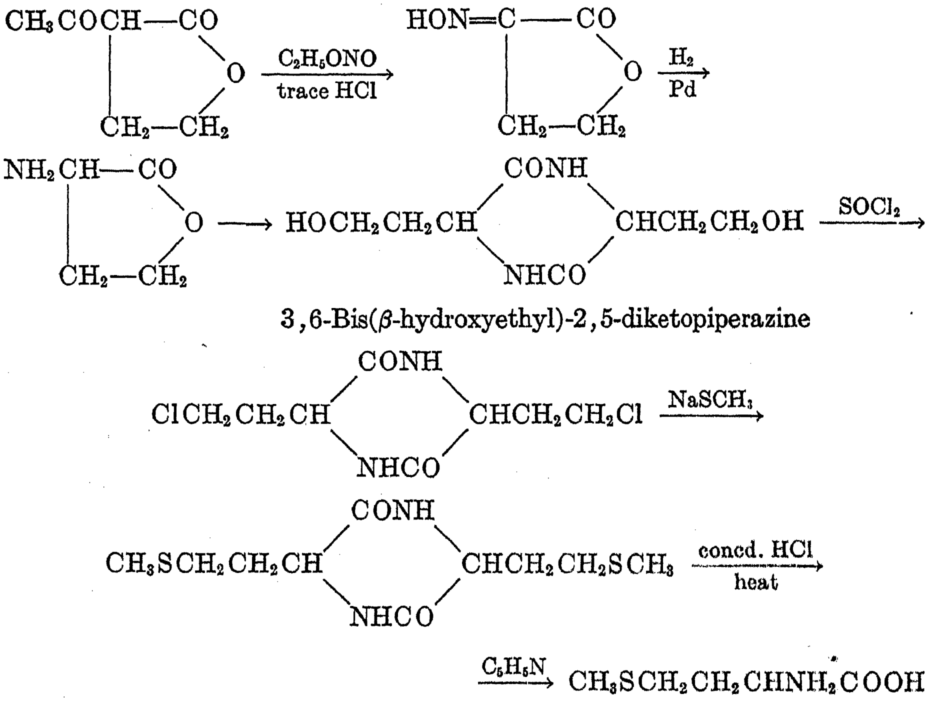


α-Aceto-γ-butyrolactone methyl chloride



Hydroxyproline

(*2*) *Methionine:* Snyder *et al.* (285, 286, 287) used α-aceto-γ-butyrolactone from ethylene oxide and acetoacetic ester to prepare methionine, essentially as follows:



3,6-Bis(β-hydroxyethyl)-2,5-diketopiperazine

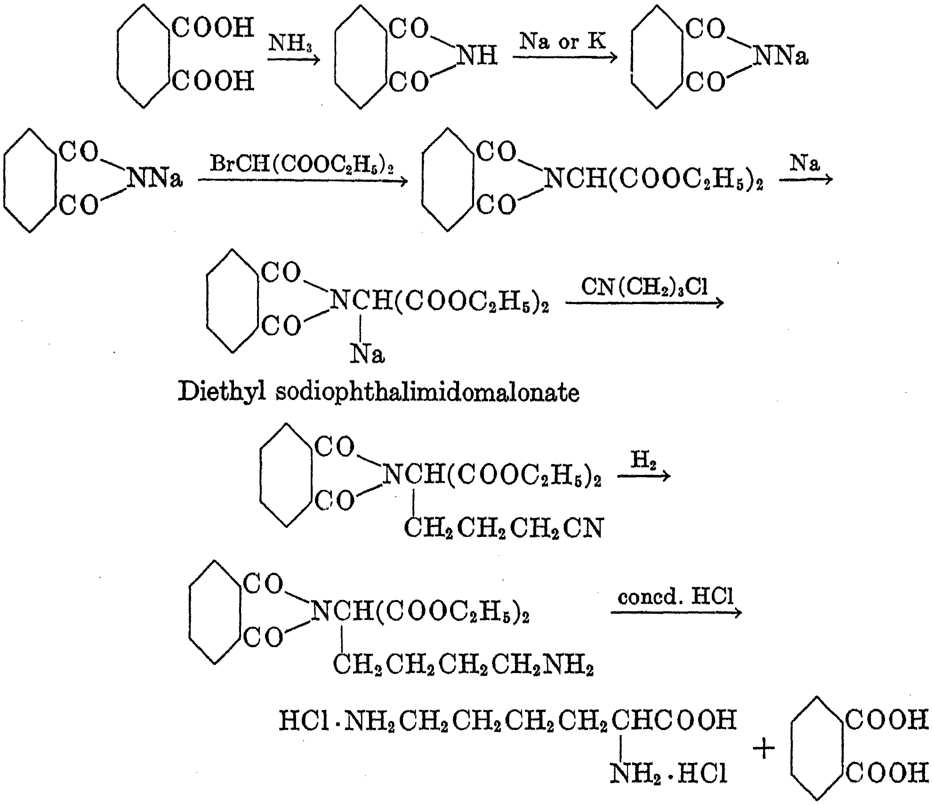
Methionine

E. ALKYLATION OF AMINOMALONIC ACIDS

1. Phthalimidomalonic ester

(a) Lysine

In 1889, Gabriel and Kroseberg (138) prepared glycine by treating chloroacetic ester with potassium phthalimide and hydrolyzing the resulting phthalimidoacetic ester (*cf.* Section III,A,3). Sørensen (292) used an interesting modification of this procedure for the synthesis of lysine (241, 293).

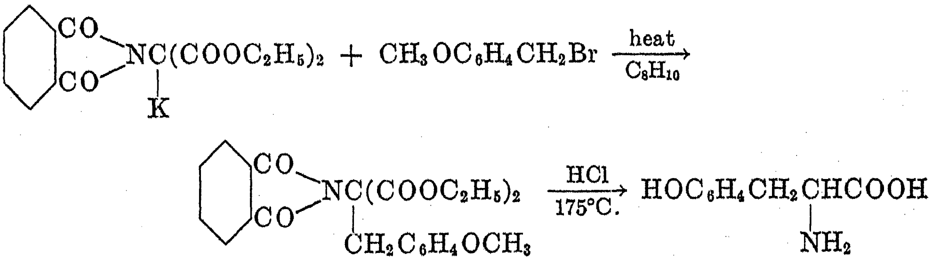


Diethyl sodiophthalimidomalonate

Lysine dihydrochloride

(b) Phenylalanine and tyrosine

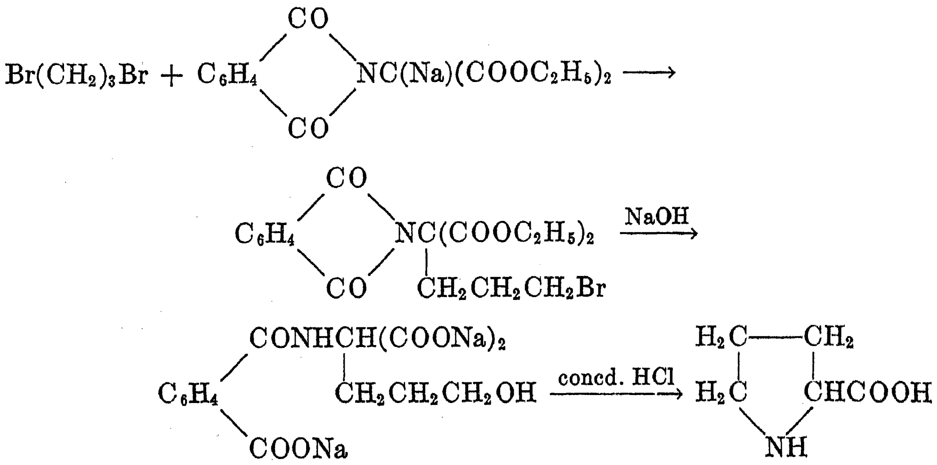
Sørensen (293) used the sodium phthalimidomalonic ester method for the synthesis of phenylalanine from benzyl chloride, while Stephen and Weizmann (298) synthesized tyrosine by the same general method from *p*-methoxybenzyl bromide.



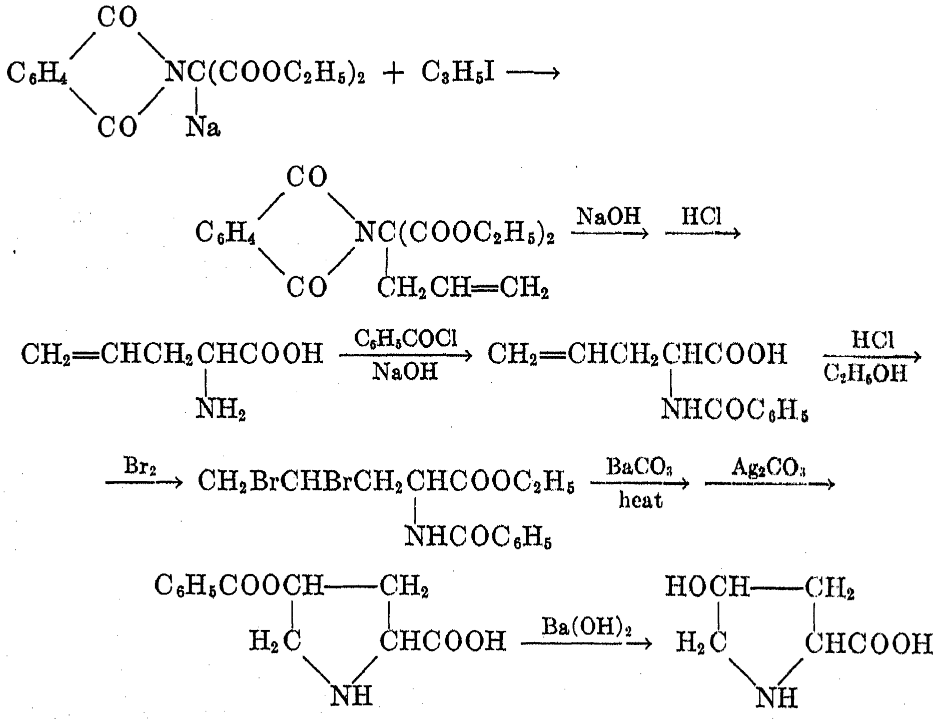
Tyrosine

(c) Proline and hydroxyproline

These amino acids were synthesized as follows (294, 297):



Proline

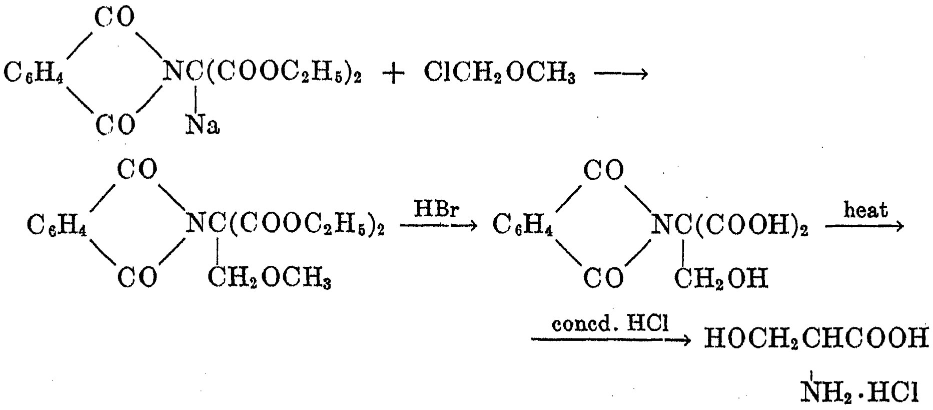


Hydroxyproline

The migration of the benzoyl group from nitrogen to oxygen is to be noted.

(d) Serine and β-hydroxynorvaline

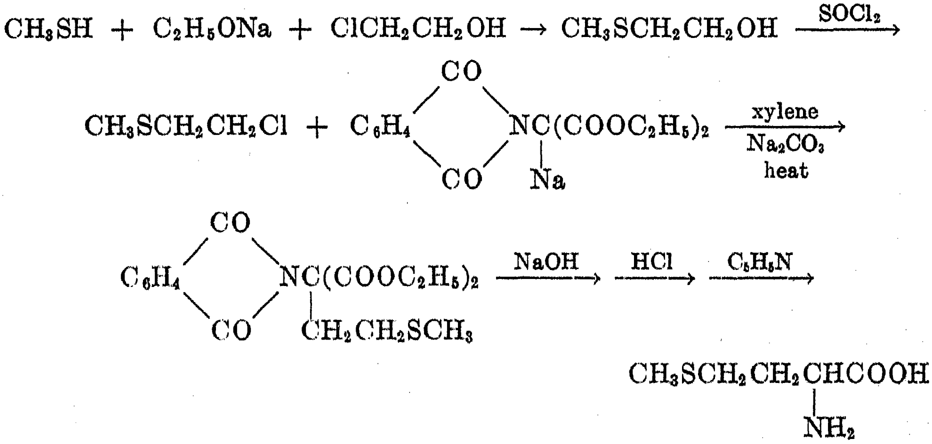
Serine has been synthesized from chloromethyl ether in the absence of a solvent (216, 231).



β-Hydroxynorvaline (3) has been prepared in a similar fashion from α-chloro-*n*-propyl ethyl ether.

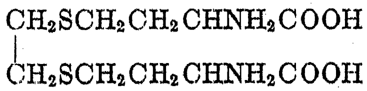
(e) Methionine and cystine

Barger and Weichselbaum (20; *cf.* 51) used Sørensen's method to synthesize methionine.



Methionine

Snyder *et al.* (288) obtained ω, ω′-bimethionine as a by-product of the Barger-Weichselbaum method.

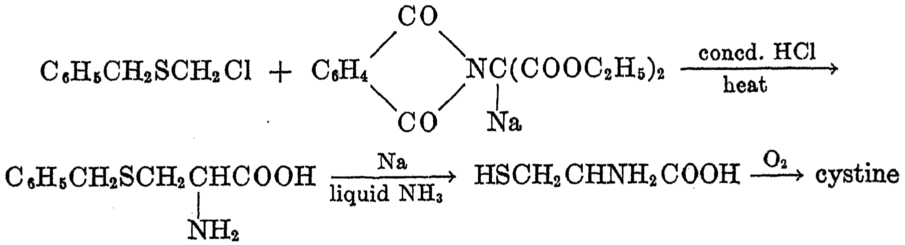


$$\begin{array}{l} CH_2SCH_2CH_2CHNH_2COOH \\ | \\ CH_2SCH_2CH_2CHNH_2COOH \end{array}$$

ω,ω′-Bimethionine

Wood and du Vigneaud (340) prepared cystine from chloromethyl benzyl sulfide.

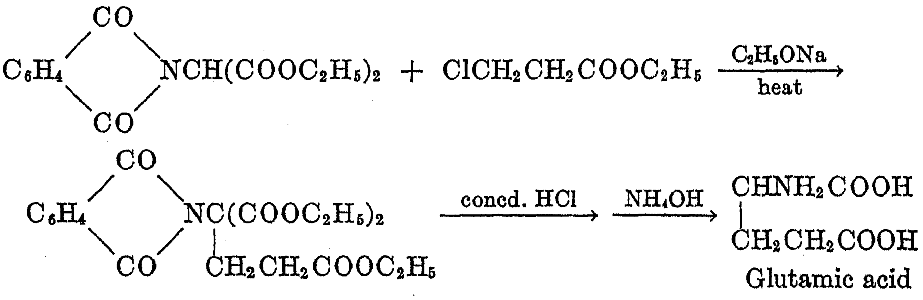
$$C_6H_5CH_2SH + (HCHO)_n + HCl \longrightarrow C_6H_5CH_2SCH_2Cl$$



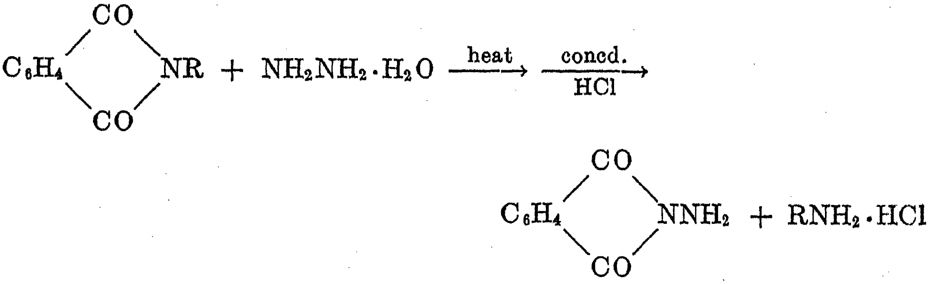
Kuhn and Quadbeck (195) synthesized lanthionine from dichlorodimethyl sulfide.

(f) Glutamic acid and aspartic acid

Dunn and Smart (98) used the Sørensen method for the synthesis of aspartic acid from chloroacetic ester, while Marvel and Stoddard (224) prepared glutamic acid from ethyl β-chloropropionate or from methyl acrylate.



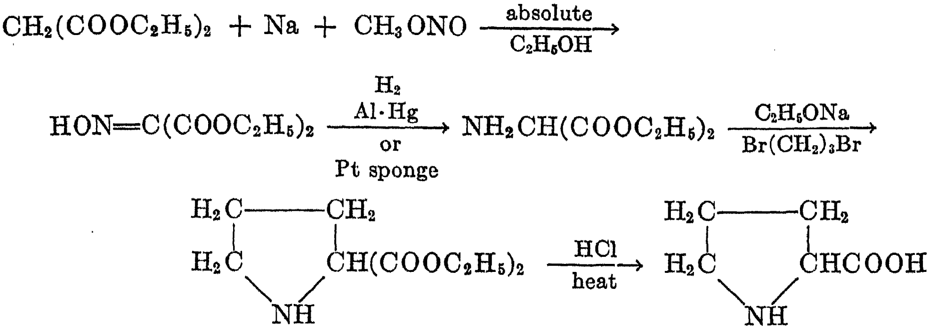
One of the chief hindrances to the use of the Gabriel–Sørensen method is the difficulty of splitting off the phthalic acid. King and Robinson (180) have facilitated this reaction by the use of hydrazine.



2. Aminomalonic ester

(a) Proline

Putochin (251) apparently was the first to use aminomalonic ester for the synthesis of an amino acid.



$$\mathrm{CH_2(COOC_2H_5)_2 + Na + CH_3ONO \xrightarrow[C_2H_5OH]{\text{absolute}}}$$

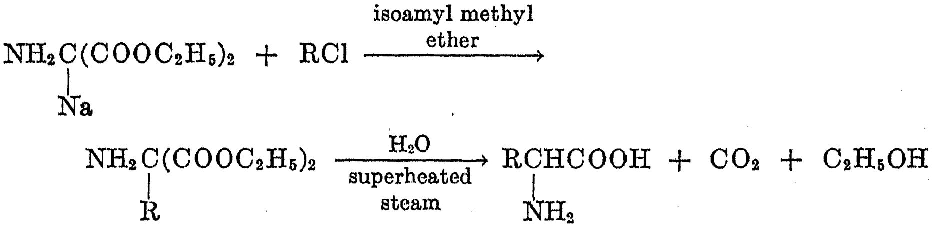
$$\mathrm{HON{=}C(COOC_2H_5)_2 \xrightarrow[\text{or Pt sponge}]{H_2\ Al\cdot Hg} NH_2CH(COOC_2H_5)_2 \xrightarrow[Br(CH_2)_3Br]{C_2H_5ONa}}$$

Proline
(25 per cent yield)

Aminomalonic acid was first obtained by Baeyer (17) by reduction of nitrosomalonic acid, but it was not readily prepared until 1880 when Conrad and Bischoff (78) synthesized nitrosomalonic ester from diethyl sodiomalonate and nitrous acid. Nitrosomalonate can be reduced by Raney nickel (203).

(b) Glycine, leucine, and phenylalanine

These amino acids were synthesized by Locquin and Cerchez (211, 212, 213) from aminomalonic ester.



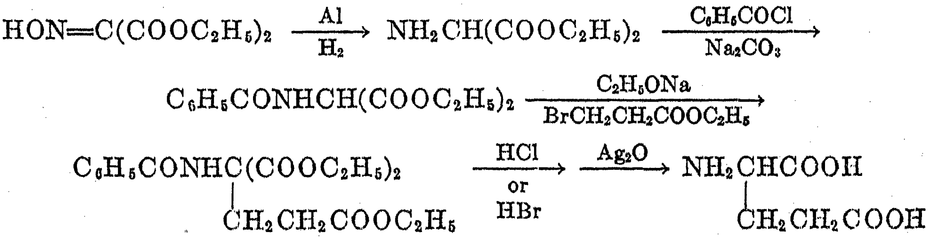
$$\mathrm{NH_2\underset{\substack{|\\ Na}}{C}(COOC_2H_5)_2 + RCl \xrightarrow{\text{isoamyl methyl ether}}}$$

$$\mathrm{NH_2\underset{\substack{|\\ R}}{C}(COOC_2H_5)_2 \xrightarrow[\text{superheated steam}]{H_2O} RCHCOOH\!\!\underset{\substack{|\\ NH_2}}{} + CO_2 + C_2H_5OH}$$

3. *Benzoylaminomalonic ester*

(a) Glutamic acid, aspartic acid, glycine, alanine, leucine, valine, and phenylalanine

Dunn, Redemann, and their coworkers introduced diethyl benzamidomalonate as a general reagent for the preparation of α-amino acids (99, 243, 255). Good to excellent yields were obtained.



$$\mathrm{HON{=}C(COOC_2H_5)_2 \xrightarrow[H_2]{Al} NH_2CH(COOC_2H_5)_2 \xrightarrow[Na_2CO_3]{C_6H_5COCl}}$$

$$\mathrm{C_6H_5CONHCH(COOC_2H_5)_2 \xrightarrow[BrCH_2CH_2COOC_2H_5]{C_2H_5ONa}}$$

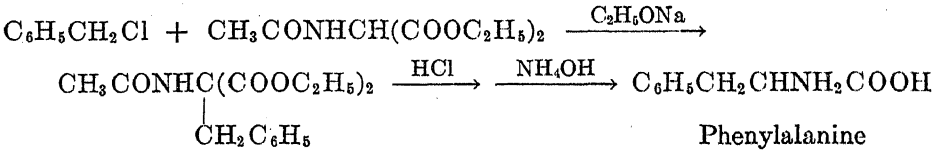
$$\mathrm{C_6H_5CONH\underset{\substack{|\\ CH_2CH_2COOC_2H_5}}{C}(COOC_2H_5)_2 \xrightarrow[\text{or HBr}]{HCl} \xrightarrow{Ag_2O} NH_2\underset{\substack{|\\ CH_2CH_2COOH}}{CH}COOH}$$

Glutamic acid
(52 per cent yield)

4. Acetylaminomalonic ester

(a) Leucine, norleucine, and phenylalanine

Dakin (87) treated acetylaminomalonic ester from aminomalonic ester and acetic anhydride with isobutylene oxide to prepare γ-hydroxyleucine, which may be a constituent of proteins, and proposed an analogous synthesis of hydroxyproline from epichlorohydrin. Snyder *et al.* (289) and Albertson *et al.* (7, 10) have recently prepared phenylalanine and leucine in from 30 to 60 per cent yields by the alkylation of diethyl sodioacetylaminomalonate.

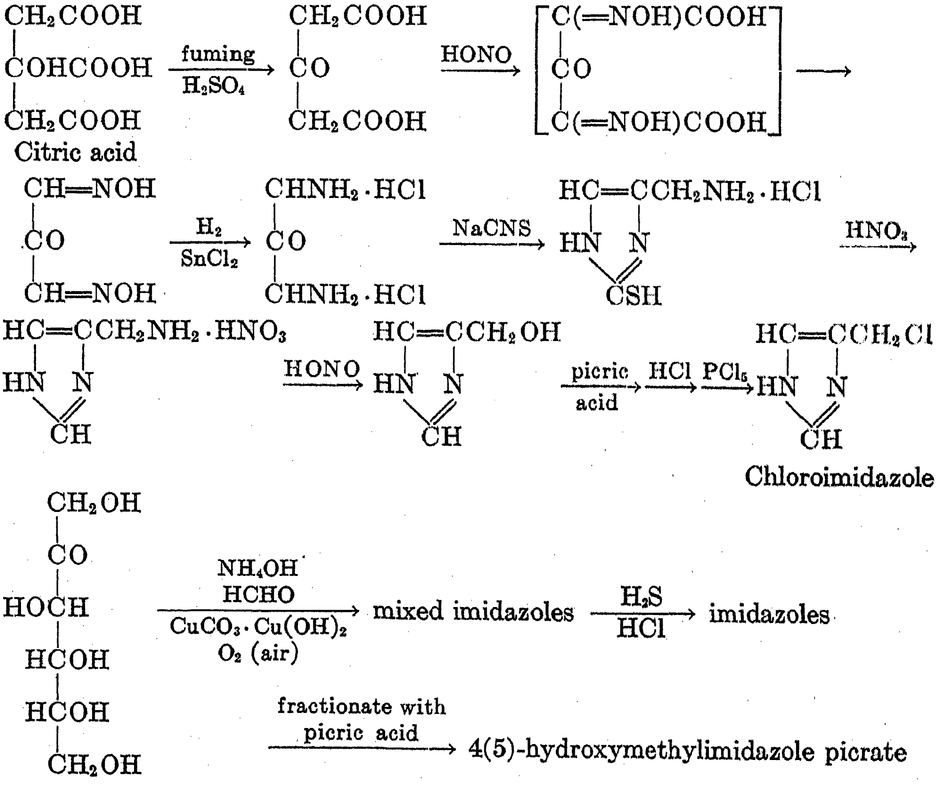


$$C_6H_5CH_2Cl + CH_3CONHCH(COOC_2H_5)_2 \xrightarrow{C_2H_5ONa}$$
$$\underset{\displaystyle CH_2C_6H_5}{CH_3CONHC(COOC_2H_5)_2} \xrightarrow{HCl} \xrightarrow{NH_4OH} \underset{\text{Phenylalanine}}{C_6H_5CH_2CHNH_2COOH}$$

Snyder *et al.* (289) were unable to prepare isoleucine or valine from *sec*-butyl bromide and isopropyl bromide, respectively, i.e., secondary halides are of little use in any variation of the Gabriel–Sørensen method.

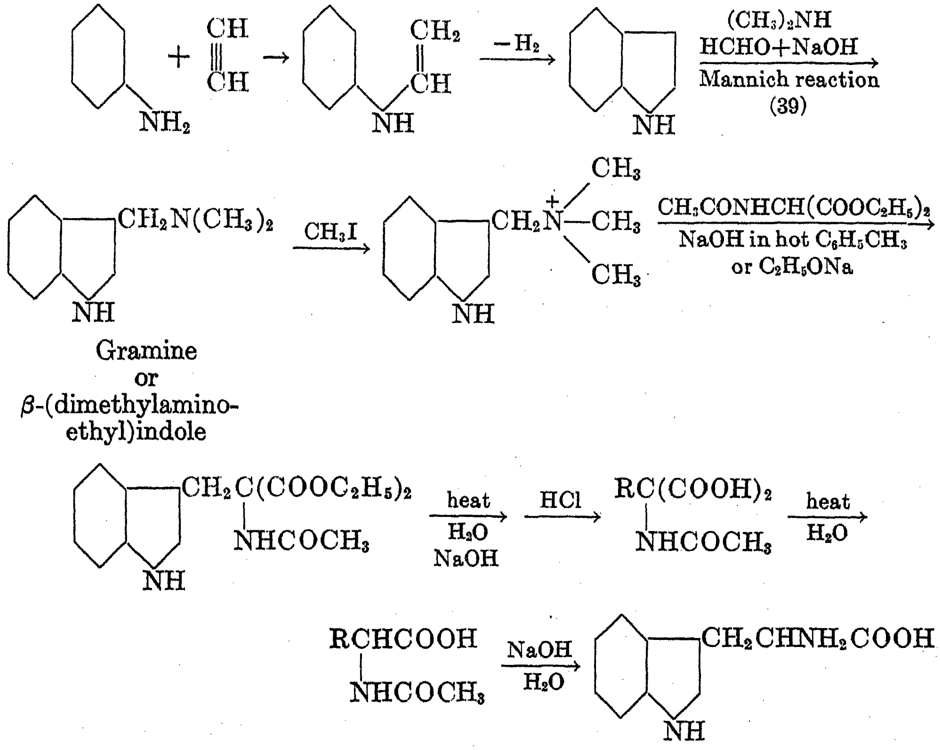
(b) Histidine

Albertson and Archer (7) synthesized histidine by the alkylation of acetylaminomalonic ester with 4(or 5)-chloroimidazole. The latter was prepared from citric acid (188) or from fructose (89, 328, 329), as indicated below:



(c) Tryptophan

Snyder *et al.* (164, 290, 291) and Albertson *et al.* (8, 9) prepared tryptophan from aniline *via* indole (218) and gramine (194) in large quantities and excellent yield.



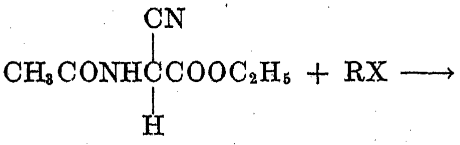
Gramine
or
β-(dimethylamino-
ethyl)indole

Tryptophan

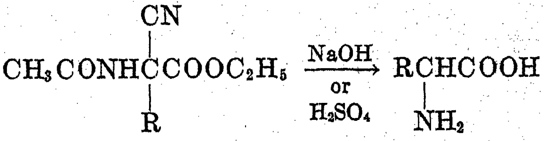
5. *Acetylaminocyanoacetic ester*

(a) Valine, methionine, phenylalanine, tryptophan, histidine, etc.

Albertson and Tuller (10) have found that acetamidocyanoacetic ester can be used as a general method for preparing amino acids. The yields varied from 60 to 80 per cent.



$$CH_3CONH\overset{CN}{\underset{H}{C}}COOC_2H_5 + RX \longrightarrow$$



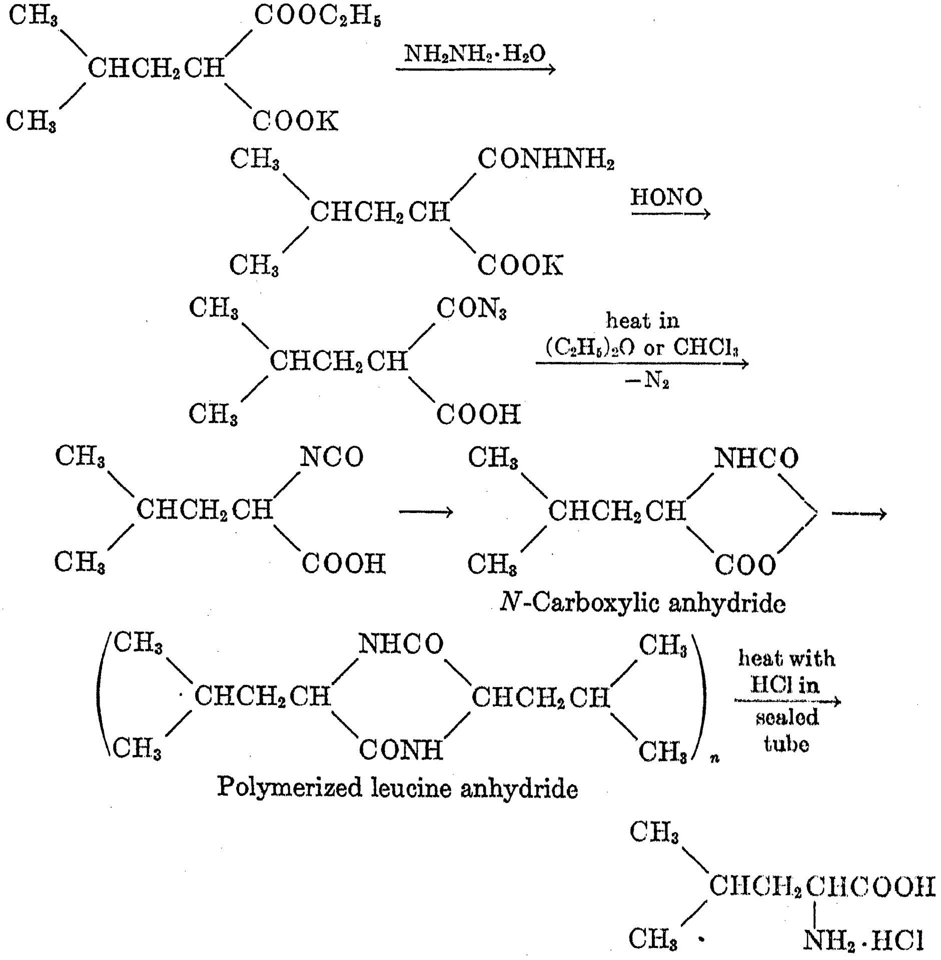
$$CH_3CONH\overset{CN}{\underset{R}{C}}COOC_2H_5 \xrightarrow[H_2SO_4]{NaOH\ \text{or}} R\underset{NH_2}{C}HCOOH$$

F. AZIDE SYNTHESIS

1. *Potassium ethyl malonate: Curtius reaction*

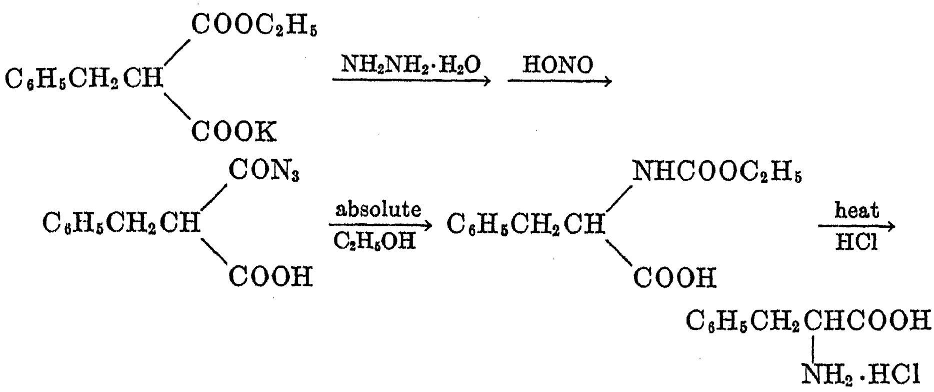
(a) Alanine, valine, leucine, and glycine

Curtius and Sieber (83, 84, 85) found that one of the carboxyl groups of malonic acid could be converted into an amino group by way of the azide. Thus, substituted malonic acids should be sources of α-amino acids. This method may be of special value when bromination of substituted malonic acids cannot be carried out owing to the reactivity of the substituent group (*cf.* Section III,A,2).


$NH_2NH_2 \cdot H_2O$
HONO
heat in $(C_2H_5)_2O$ or $CHCl_3$
$-N_2$
N-Carboxylic anhydride
Polymerized leucine anhydride
heat with HCl in sealed tube

(b) Phenylalanine

If the azide is treated with absolute alcohol, the reaction takes a slightly different course and the amino acid is formed *via* the urethan.



Azides are toxic and explosive.

2. *Cyanoacetic ester: Curtius reaction*

(a) Glycine

Sah (261) proposed the use of the azides of cyanoacetic ester for the synthesis of α-amino acids. The paper which has come to the author's attention, however, only describes the synthesis of glycine, although other "paper" syntheses are mentioned.

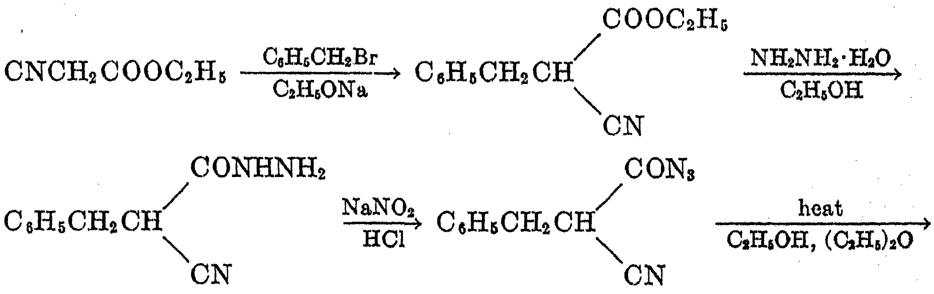
$$\mathrm{CNCH_2COOC_2H_5} \xrightarrow{\mathrm{NH_2NH_2\cdot H_2O}} \mathrm{CNCH_2CONHNH_2} \xrightarrow[\mathrm{HCl}]{\mathrm{NaNO_2}}$$

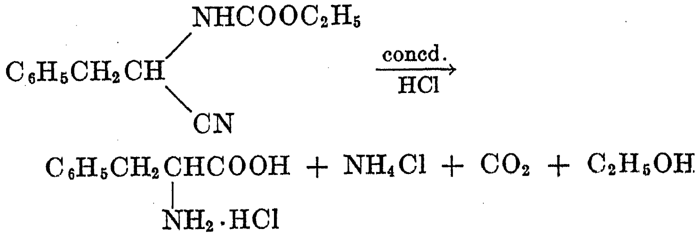
$$\mathrm{CNCH_2CON_3} \xrightarrow[\mathrm{(C_2H_5)_2O}]{\mathrm{C_2H_5OH}} \mathrm{CNCH_2NHCOOC_2H_5} \xrightarrow[\text{heat}]{\mathrm{Ba(OH)_2}}$$

$$\xrightarrow{\mathrm{H_2SO_4}} \underset{\text{Glycine}}{\mathrm{NH_2CH_2COOH}}$$

(b) Leucine, valine, norleucine, tyrosine, and phenylalanine

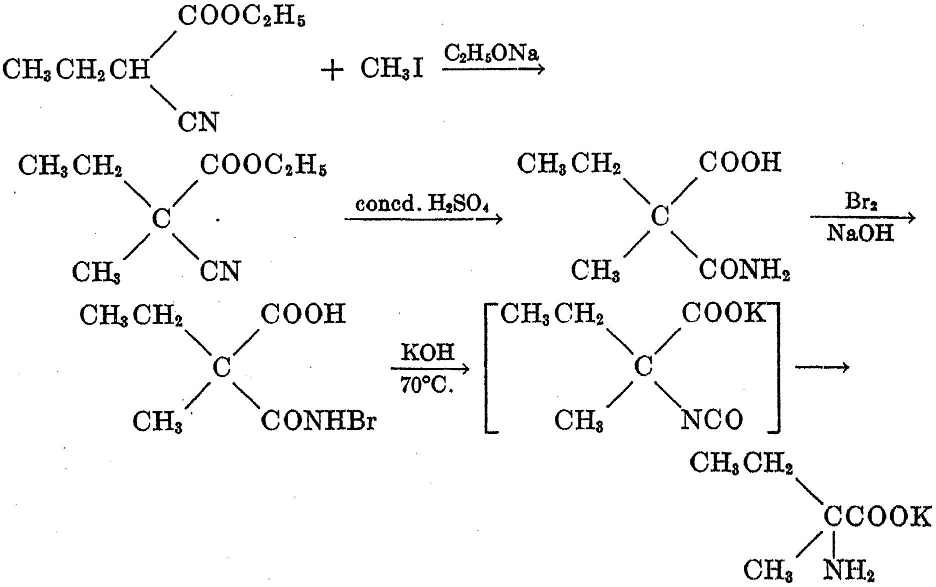
Darapsky *et al.* (88) also used the hydrazide of cyanoacetic ester for the preparation of amino acids. Their work has been substantiated and extended by Gagnon *et al.* (139), who found, however, that the method is inoperative for the preparation of diamino acids.





3. Cyanoacetic ester: Hofmann rearrangement

Li *et al.* (206) made an interesting use of the well-known Hofmann rearrangement to synthesize an α-amino acid (isovaline).

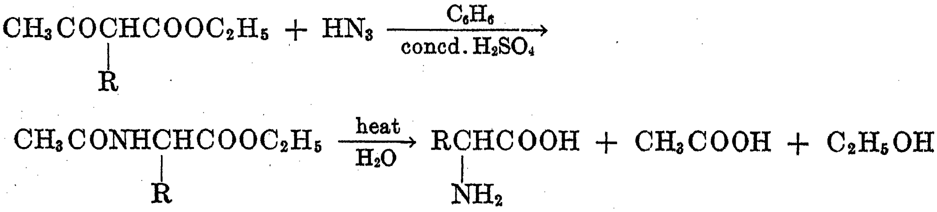


4. Hydrazoic acid: Schmidt reaction

This interesting reaction is essentially a Beckmann–Curtius rearrangement, the net result being the insertion of an imine residue.

(a) Glycine, phenylalanine, and aspartic acid

Schmidt prepared these amino acids in from 80–98 per cent yields (272) by the action of hydrazoic acid on acetoacetic ester.

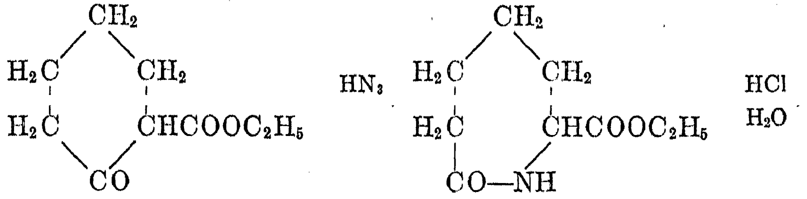


$$CH_3CO\underset{\substack{|\\ R}}{C}HCOOC_2H_5 + HN_3 \xrightarrow[\text{concd. }H_2SO_4]{C_6H_6}$$

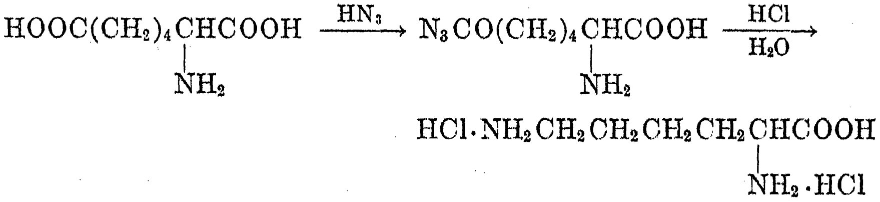
$$CH_3CONH\underset{\substack{|\\ R}}{C}HCOOC_2H_5 \xrightarrow[H_2O]{\text{heat}} R\underset{\substack{|\\ NH_2}}{C}HCOOH + CH_3COOH + C_2H_5OH$$

(b) Lysine

Adamson (5) observed that when α-amino dicarboxylic acids are submitted to the Schmidt hydrazoic acid reaction, the carboxyl group remote from the α-NH_2 is replaced by NH_2.



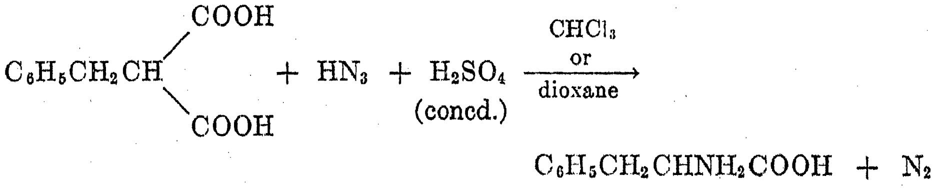
Ethyl cyclohexanone-2-carboxylate



$$HOOC(CH_2)_4\underset{NH_2}{CH}COOH \xrightarrow{HN_3} N_3CO(CH_2)_4\underset{NH_2}{CH}COOH \xrightarrow[H_2O]{HCl} HCl\cdot NH_2CH_2CH_2CH_2CH_2\underset{NH_2\cdot HCl}{CH}COOH$$

(c) Phenylalanine

Briggs *et al.* (61) used the Schmidt reaction to prepare phenylalanine from benzylmalonic acid.



$$C_6H_5CH_2CH\begin{matrix}\diagup COOH\\ \diagdown COOH\end{matrix} + HN_3 + \underset{\text{(concd.)}}{H_2SO_4} \xrightarrow[\text{dioxane}]{CHCl_3 \text{ or}} C_6H_5CH_2CHNH_2COOH + N_2$$

Phenylalanine
(16 per cent yield)

It should be recalled that hydrazoic acid is toxic and explosive.

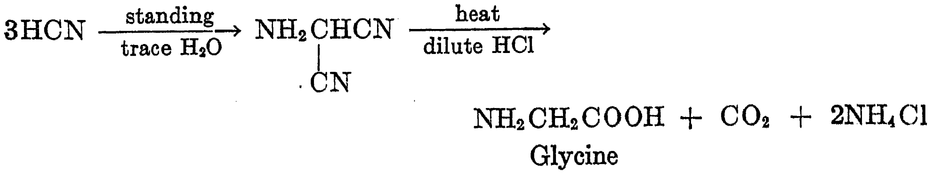
G. MISCELLANEOUS

A number of syntheses have been proposed which have been used to prepare only one or two amino acids or which are only applicable to specific amino acids. These will now be briefly considered according to the amino acids to which they have been applied.

1. *Alanine and glycine*

(a) Glycine from cyanide

Lange (199) and Emmerling (107) prepared small quantities of glycine from hydrogen cyanide and cyanogen, respectively.



$$3HCN \xrightarrow[\text{trace } H_2O]{\text{standing}} \underset{\displaystyle \cdot CN}{NH_2CHCN} \xrightarrow[\text{dilute HCl}]{\text{heat}} \underset{\text{Glycine}}{NH_2CH_2COOH} + CO_2 + 2NH_4Cl$$

$$(CN)_2 + 6HI + 2H_2O \xrightarrow{\text{heat}} HI\cdot NH_2CH_2COOH + NH_4I + 2I_2$$

More recently Gluud *et al.* (141) have successfully used cyanoformic acid or ester (141) to make glycine.

$$CNCOOH \xrightarrow[\text{electrolytic}]{H_2} NH_2CH_2COOH$$

$$CNCOOC_2H_5 + (CH_3CO)_2O \xrightarrow[H_2]{Ni} CH_3CONHCH_2COOC_2H_5 \xrightarrow{HCl} HCl\cdot NH_2CH_2COOH$$

Alderson (11) has replaced the sulfonic acid group of aminomethylsulfonic acid with —CN and hydrolyzed the resulting product to yield glycine.

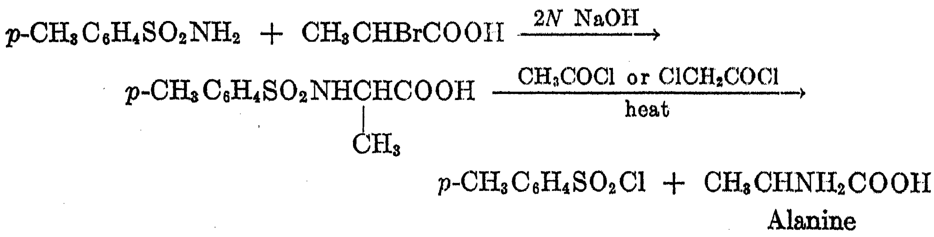
$$NH_2CH_2SO_2OH + NaOH + NaCN \xrightarrow{\text{heat}} NH_2CH_2CN \xrightarrow[\text{heat}]{HCl}$$

$$HCl\cdot NH_2CH_2COOH \xrightarrow{C_5H_5N} \underset{\text{Glycine}}{NH_2CH_2COOH} + C_5H_5N\cdot HCl$$

Pyridine hydrochloride is soluble in absolute alcohol.

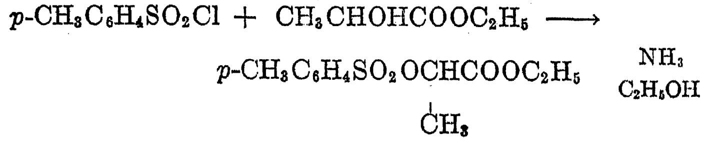
(b) Glycine and alanine by metathesis

Schroeter (276, 277) used what amounts to double decomposition between an acid amide (*p*-toluenesulfonamide) and an acid chloride to synthesize alanine and glycine. This is a most suggestive paper, and the process described should avoid many of the by-products formed by direct amination of α-halogen acids (*cf.* Section III,A).

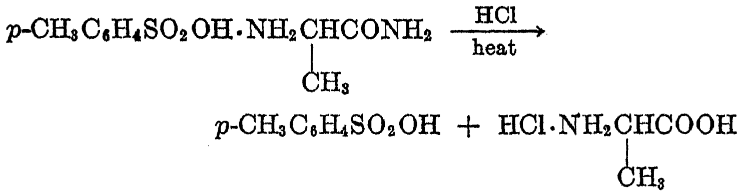


$$p\text{-}CH_3C_6H_4SO_2NH_2 + CH_3CHBrCOOH \xrightarrow{2N\ NaOH} p\text{-}CH_3C_6H_4SO_2NH\underset{\displaystyle CH_3}{CH}COOH \xrightarrow[\text{heat}]{CH_3COCl \text{ or } ClCH_2COCl} p\text{-}CH_3C_6H_4SO_2Cl + \underset{\text{Alanine}}{CH_3CHNH_2COOH}$$

Freudenberg and Huber (137) prepared alanine from lactic acid along somewhat similar lines.



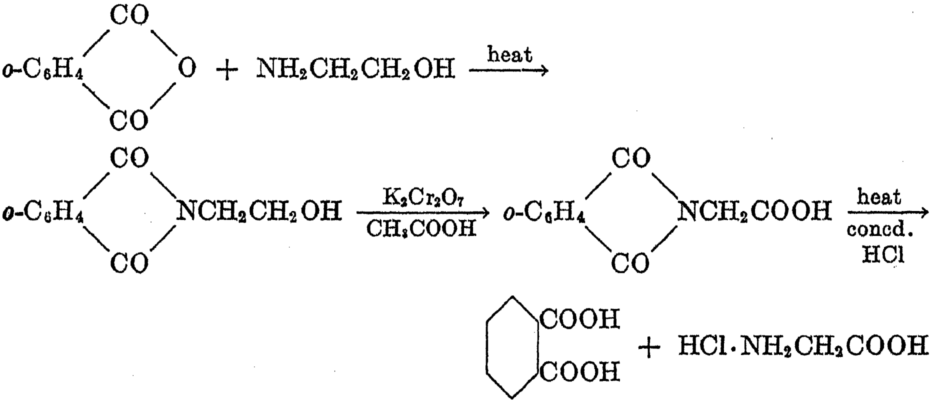
$$p\text{-}CH_3C_6H_4SO_2Cl + CH_3CHOHCOOC_2H_5 \longrightarrow p\text{-}CH_3C_6H_4SO_2O\underset{\displaystyle CH_3}{CH}COOC_2H_5 \quad \begin{matrix} NH_3 \\ C_2H_5OH \end{matrix}$$



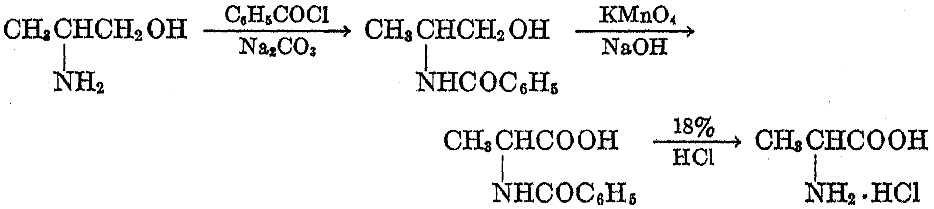
$$\mathrm{\textit{p}\text{-}CH_3C_6H_4SO_2OH\cdot NH_2\underset{\underset{\displaystyle CH_3}{|}}{C}HCONH_2} \xrightarrow[\text{heat}]{\mathrm{HCl}} \mathrm{\textit{p}\text{-}CH_3C_6H_4SO_2OH + HCl\cdot NH_2\underset{\underset{\displaystyle CH_3}{|}}{C}HCOOH}$$

(c) Glycine and alanine by oxidation of amino alcohols

Billman and Parker (36, 37) have made use of the commercially available amino alcohols for the preparation of the simpler amino acids. The yields are excellent.



Glycine hydrochloride



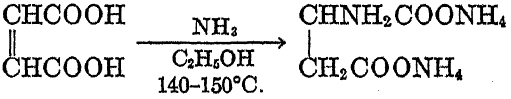
$$\mathrm{CH_3\underset{\underset{\displaystyle NH_2}{|}}{C}HCH_2OH} \xrightarrow[\mathrm{Na_2CO_3}]{\mathrm{C_6H_5COCl}} \mathrm{CH_3\underset{\underset{\displaystyle NHCOC_6H_5}{|}}{C}HCH_2OH} \xrightarrow[\mathrm{NaOH}]{\mathrm{KMnO_4}} \mathrm{CH_3\underset{\underset{\displaystyle NHCOC_6H_5}{|}}{C}HCOOH} \xrightarrow[\mathrm{HCl}]{18\%} \mathrm{CH_3\underset{\underset{\displaystyle NH_2\cdot HCl}{|}}{C}HCOOH}$$

Alanine
hydrochloride

2. Aspartic and glutamic acids

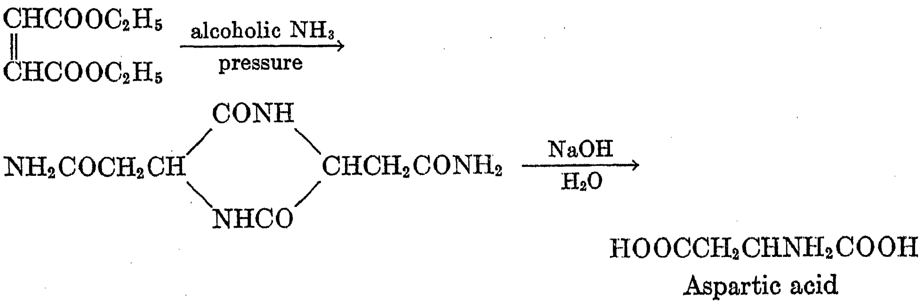
(a) Aspartic acid from maleic or fumaric acid

Engel (108) synthesized ammonium aspartate by the addition of ammonia to maleic or fumaric acid. The reaction is catalyzed by mercuric salts (110) and ammonium chloride (319).



$$\begin{array}{l}\mathrm{CHCOOH}\\ \|\\ \mathrm{CHCOOH}\end{array} \xrightarrow[\substack{\mathrm{C_2H_5OH}\\ 140\text{–}150^\circ\mathrm{C.}}]{\mathrm{NH_3}} \begin{array}{l}\mathrm{CHNH_2COONH_4}\\ |\\ \mathrm{CH_2COONH_4}\end{array}$$

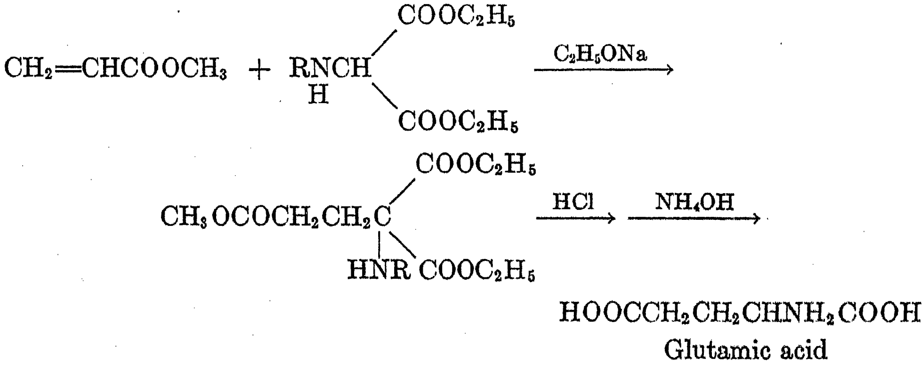
Dunn and Fox (96), using diethyl fumarate, obtained the diketopiperazine of aspartic acid amide as the intermediary product.



Wolff (339) as early as 1850 also claimed to have synthesized aspartic acid by heating ammonium maleate. The identification of aspartic acid given in Wolff's paper does not appear to be conclusive.

(b) Glutamic acid by Michael condensation

Marvel and Stoddard (224) and Snyder *et al.* (289) have synthesized glutamic acid from methyl acrylate and phthalimidomalonic ester or acetamidomalonic ester, respectively. This appears to be a Michael condensation (*cf.* 289) rather than an alkylation reaction.

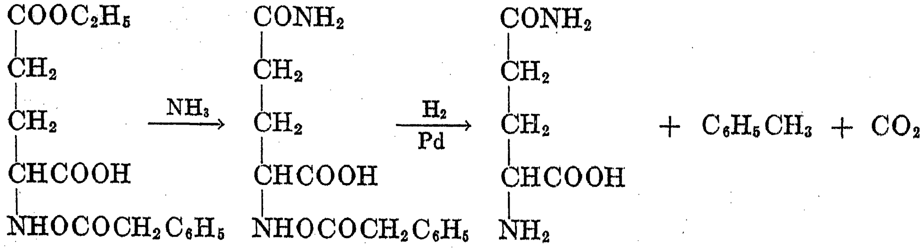


(c) Glutamine and asparagine

Bergmann *et al.* (34, 35, 235) synthesized asparagine and glutamine from the corresponding carbobenzoxy amino acids.

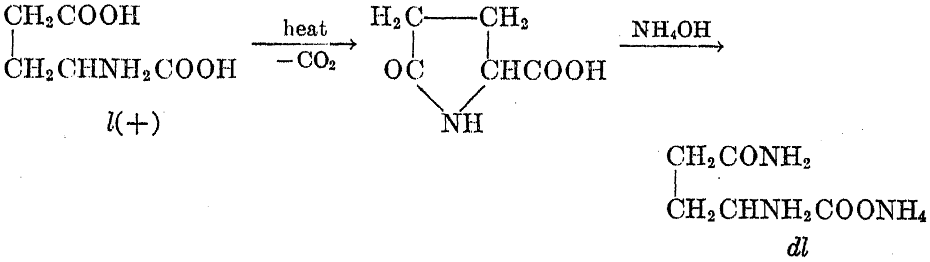
$$ClCOCl + C_6H_5CH_2OH \longrightarrow C_6H_5CH_2OCOCl$$

Benzylcarbonyl chloride



$$\begin{array}{l}COOC_2H_5\\ |\\ CH_2\\ |\\ CH_2\\ |\\ CHCOOH\\ |\\ NHOCOCH_2C_6H_5\end{array} \xrightarrow{NH_3} \begin{array}{l}CONH_2\\ |\\ CH_2\\ |\\ CH_2\\ |\\ CHCOOH\\ |\\ NHOCOCH_2C_6H_5\end{array} \xrightarrow[Pd]{H_2} \begin{array}{l}CONH_2\\ |\\ CH_2\\ |\\ CH_2\\ |\\ CHCOOH\\ |\\ NH_2\end{array} + C_6H_5CH_3 + CO_2$$

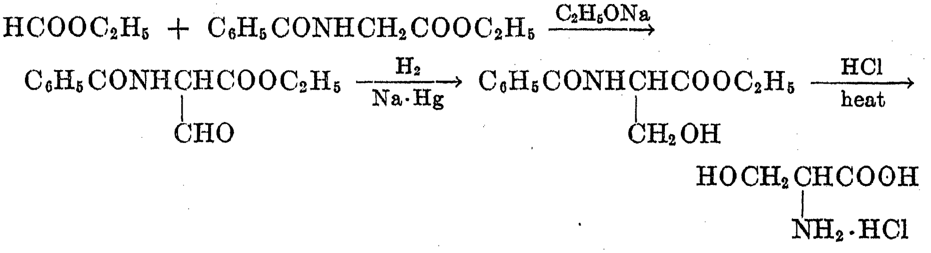
Lichtenstein (207) has synthesized glutamine from glutamic acid *via* pyrrolidonecarboxylic acid. Considerable quantities of ammonium pyrrolidonecarboxylate are also formed.



3. *Serine and threonine*

(a) Serine from ethyl hippurate

As long ago as 1904, Erlenmeyer and Stoop (115) synthesized serine from ethyl formate and hippuric acid ester.

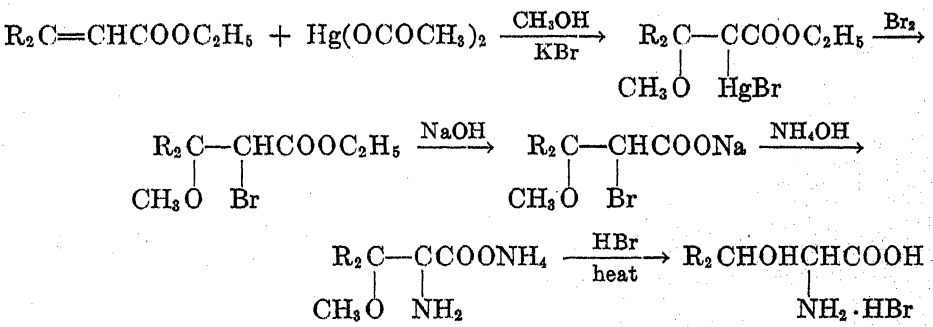


$$HCOOC_2H_5 + C_6H_5CONHCH_2COOC_2H_5 \xrightarrow{C_2H_5ONa} \underset{\displaystyle CHO}{C_6H_5CONHCHCOOC_2H_5} \xrightarrow[Na\cdot Hg]{H_2} \underset{\displaystyle CH_2OH}{C_6H_5CONHCHCOOC_2H_5} \xrightarrow[heat]{HCl} \underset{\displaystyle NH_2\cdot HCl}{HOCH_2CHCOOH}$$

Serine hydrochloride

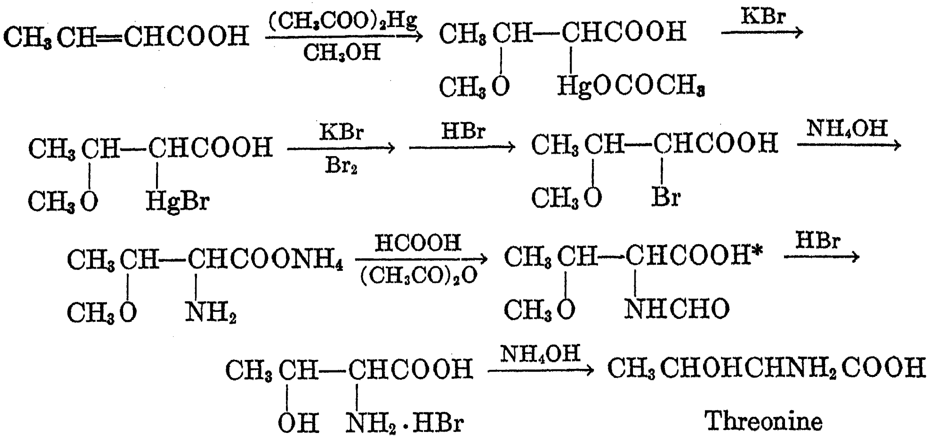
(b) Serine and threonine by the addition of mercury salts to α,β-olefincarboxylic esters

Schrauth and Geller (275) found that when mercuric salts in alcohol were allowed to react with α,β-olefincarboxylic esters, Hg^{++} was introduced into the α-position and the alkyl group of the alcohol in the β-position.



$$R_2C{=}CHCOOC_2H_5 + Hg(OCOCH_3)_2 \xrightarrow[KBr]{CH_3OH} \underset{\displaystyle CH_3O\quad HgBr}{R_2C{-}CCOOC_2H_5} \xrightarrow{Br_2} \underset{\displaystyle CH_3O\quad Br}{R_2C{-}CHCOOC_2H_5} \xrightarrow{NaOH} \underset{\displaystyle CH_3O\quad Br}{R_2C{-}CHCOONa} \xrightarrow{NH_4OH} \underset{\displaystyle CH_3O\quad NH_2}{R_2C{-}CCOONH_4} \xrightarrow[heat]{HBr} \underset{\displaystyle NH_2\cdot HBr}{R_2CHOHCHCOOH}$$

Abderhalden and Heyns (3) and Carter and West (69) have used this method to prepare threonine, while Carter (268) and Botvinnick *et al.* (52) employed it to make serine and β-hydroxynorvaline, respectively.



$$CH_3CH{=}CHCOOH \xrightarrow[CH_3OH]{(CH_3COO)_2Hg} CH_3\underset{CH_3O}{CH}-\underset{HgOCOCH_3}{C}HCOOH \xrightarrow{KBr}$$

$$CH_3\underset{CH_3O}{CH}-\underset{HgBr}{C}HCOOH \xrightarrow[Br_2]{KBr} \xrightarrow{HBr} CH_3\underset{CH_3O}{CH}-\underset{Br}{C}HCOOH \xrightarrow{NH_4OH}$$

$$CH_3\underset{CH_3O}{CH}-\underset{NH_2}{C}HCOONH_4 \xrightarrow[(CH_3CO)_2O]{HCOOH} CH_3\underset{CH_3O}{CH}-\underset{NHCHO}{C}HCOOH^{*} \xrightarrow{HBr}$$

$$CH_3\underset{OH}{CH}-\underset{NH_2\cdot HBr}{C}HCOOH \xrightarrow{NH_4OH} \underset{\text{Threonine}}{CH_3CHOHCHNH_2COOH}$$

* This step is used to separate *dl-O*-methylformylthreonine from the relatively more soluble *dl-O*-methylformylallothreonine (331).

(c) Serine and threonine by halogenation of olefinic acids

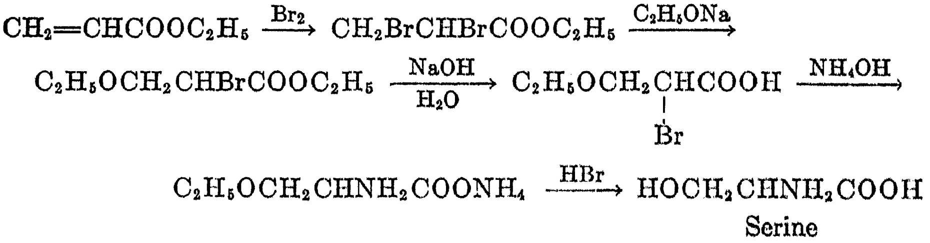
Burch (65) synthesized a mixture of threonine and allothreonine by the action of hypochlorous acid on crotonic acid.

$$CH_3CH{=}CHCOOH \xrightarrow{HOCl} CH_3CHOHCHClCOOH \xrightarrow[CH_3OH]{NH_3}$$

$$CH_3CHOHCHNH_2COOH$$

Threonine and allothreonine

Wood and du Vigneaud (341) prepared serine by bromination of acrylic ester.



$$CH_2{=}CHCOOC_2H_5 \xrightarrow{Br_2} CH_2BrCHBrCOOC_2H_5 \xrightarrow{C_2H_5ONa}$$

$$C_2H_5OCH_2CHBrCOOC_2H_5 \xrightarrow[H_2O]{NaOH} C_2H_5OCH_2\underset{Br}{C}HCOOH \xrightarrow{NH_4OH}$$

$$C_2H_5OCH_2CHNH_2COONH_4 \xrightarrow{HBr} \underset{\text{Serine}}{HOCH_2CHNH_2COOH}$$

4. Tyrosine, halogenated tyrosines, and thyroxine

(a) Tyrosine

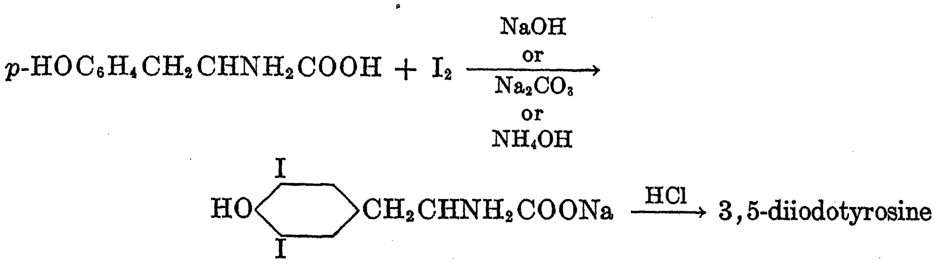
Erlenmeyer and Lipp (114) prepared tyrosine by the following reaction, which is of theoretical interest only.

$$p\text{-}H_2NC_6H_4CH_2CHNH_2COOH \xrightarrow[N_2O_3]{0°C.} p\text{-}HOC_6H_4CH_2CHNH_2COOH$$

Tyrosine

(b) Iodogorgoic acid, dibromotyrosine, and dichlorotyrosine

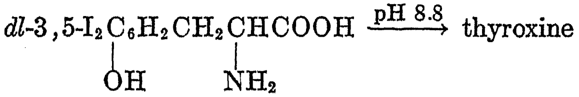
Wheeler and Jameson (334) synthesized diiodotyrosine in good yields by the addition of iodine to tyrosine in alkaline solution (*cf.* 266).



Zeynek (343) prepared 3,5-dibromo- and 3,5-dichloro-tyrosines by the halogenation of tyrosine in hot glacial acetic acid.

(c) Thyroxine from diiodotyrosine

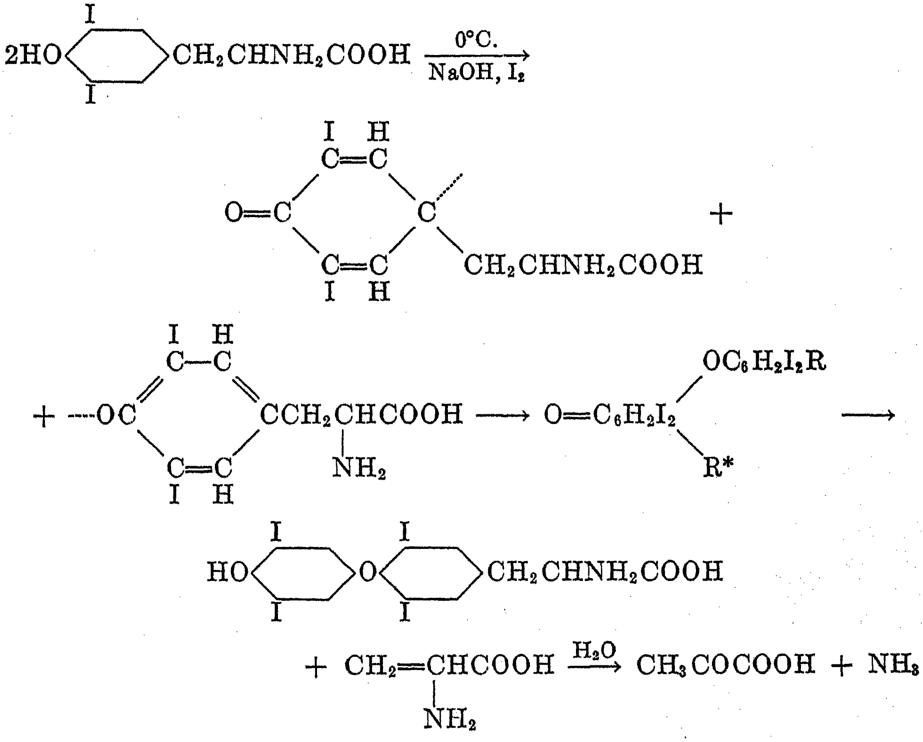
v. Mutzenbecher (234), Block (40), and others have found that small yields of thyroxine are formed from iodogorgoic acid in mildly alkaline solution (pH 8.8) at 37°C.



$$dl\text{-}3,5\text{-}\underset{\mathrm{OH}}{\mathrm{I_2C_6H_2}}\mathrm{CH_2}\underset{\mathrm{NH_2}}{\mathrm{CH}}\mathrm{COOH} \xrightarrow{\text{pH } 8.8} \text{thyroxine}$$

(15 g.) (13.5 mg.)

This reaction has been explained by Johnson (171; *cf.* 153a) as follows:

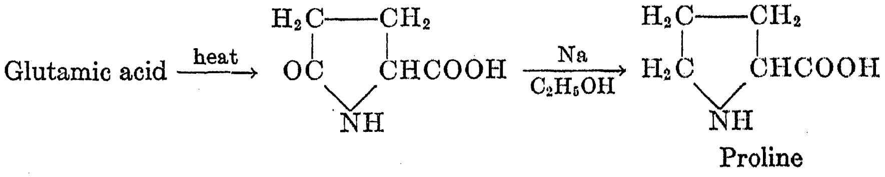


* R = $-\mathrm{CH_2CHNH_2COOH}$.

5. *Proline*

(a) Proline from pyrrolidonecarboxylic acid

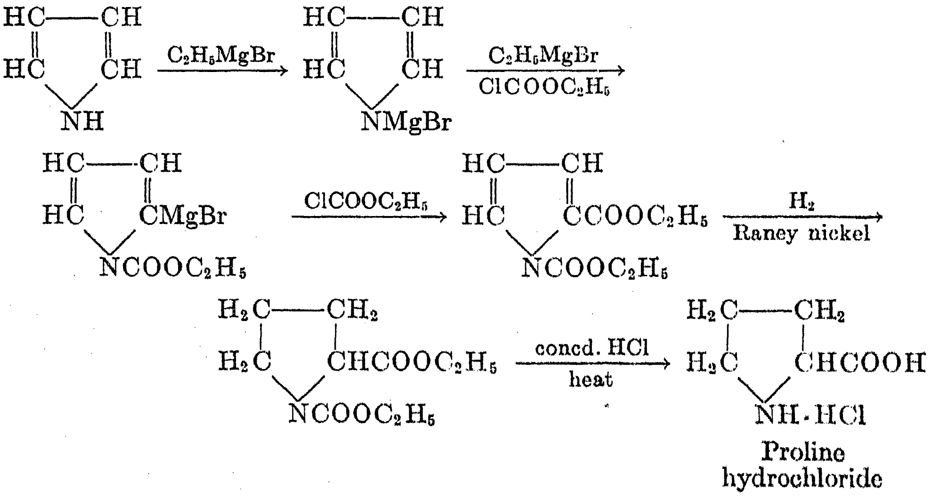
Fischer and Boehner (126) obtained proline in less than 4 per cent yield by the reduction of pyrrolidonecarboxylic acid.



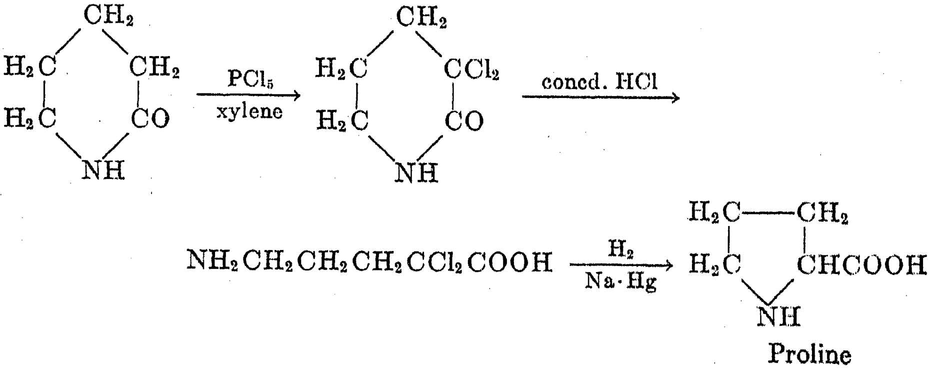
(b) Proline from pyrrole

Putokin (252) obtained proline by the reduction of pyrrolecarboxylic acid with hydrogen, using platinum or palladium oxide catalysts.

Signaigo and Adkins (282) made use of the Grignard reaction to synthesize proline from pyrrole.



Heymons (160) prepared proline from α-piperidone as follows:



6. Arginine and citrulline

(a) Arginine from ornithine

Sørensen (295, 296) in his studies on the structure of amino acids synthesized arginine from α-benzoylornithine and cyanamide.

(b) Citrulline from arginine or ornithine

Kurtz (196) prepared citrulline from ornithine copper by condensation with urea.

$$NH_2(CH_2)_3CHNH_2COOH + Cu_2O \longrightarrow$$

$$RCu \xrightarrow{NH_2CONH_2} \xrightarrow{H_2S} NH_2CONHCH_2CH_2CH_2CHNH_2COOH$$

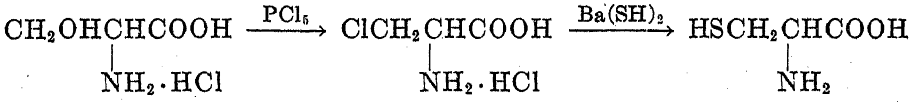
Citrulline

Fox (135) isolated citrulline after boiling arginine hydrochloride with 5.68 *N* sodium hydroxide.

7. Cysteine and methionine

(a) Cysteine from serine

Erlenmeyer (115) synthesized cysteine from serine by melting with phosphorus pentasulfide, while Fischer (127) made it from serine in small yields as follows:

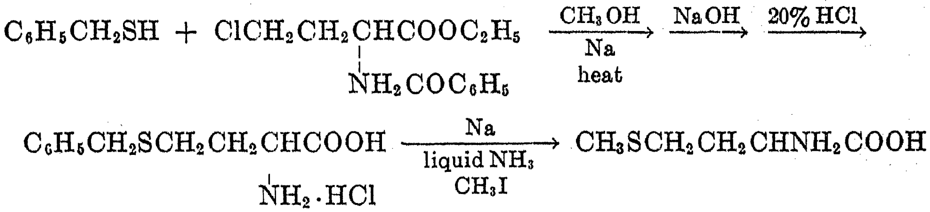


$$\underset{NH_2\cdot HCl}{CH_2OHCHCOOH} \xrightarrow{PCl_5} \underset{NH_2\cdot HCl}{ClCH_2CHCOOH} \xrightarrow{Ba(SH)_2} \underset{NH_2}{HSCH_2CHCOOH}$$

Cysteine

(b) Methionine from ethyl chloroethylhippurate

Tarver and Schmidt (305) used ethyl γ-chloro-α-benzoylaminobutyrate as the starting material for the synthesis of methionine containing radioactive sulfur.



$$C_6H_5CH_2SH + \underset{NH_2COC_6H_5}{ClCH_2CH_2CHCOOC_2H_5} \xrightarrow[\substack{Na\\heat}]{CH_3OH} \xrightarrow{NaOH} \xrightarrow{20\%\,HCl}$$

$$\underset{NH_2\cdot HCl}{C_6H_5CH_2SCH_2CH_2CHCOOH} \xrightarrow[\substack{liquid\ NH_3\\CH_3I}]{Na} CH_3SCH_2CH_2CHNH_2COOH$$

Methionine

8. β-Alanine

Although β-alanine has not been found as a protein constituent, it is widespread in nature as a portion of carnosine, pantothenic acid, etc. It is readily synthesized by the reduction of cyanoacetic esters (259, 276, 332).

$$CNCH_2COOC_2H_5 \xrightarrow{H_2} NH_2CH_2CH_2COOC_2H_5 \xrightarrow{H_2O} NH_2CH_2CH_2COOH$$

β-Alanine

H. GENERAL

Benedict (29) found that aniline can replace pyridine in the removal of hydrochloric acid from amino acid hydrochlorides.

Although acylation plus fractionation of the acylated amino acids with brucine, strychnine, etc., is usually employed in the resolution of amino acids (153, 204, etc.), Behrens *et al.* (27, 28) have resolved *dl*-phenylalanine and *dl*-alanine by means of enzymes. Duschinsky (100), however, found that it is possible to prepare *d*- and *l*-histidine from synthetic *dl*-histidine by fractional crystallization of the hydrochlorides. The reader is referred to the original paper for the details of this unique resolution in the amino acid field.

IV. REFERENCES

(1) ABDERHALDEN, E., FROEHLICH, C., AND FUCHS, D.: Resolution of *dl*-aminocaproic acid (norleucine) into its optically active components by its formyl compound. Polypeptides of which aminocaproic acid is a component. Z. physiol. Chem. **86**, 454–68 (1913).

(2) ABDERHALDEN, E., AND HEYNS, K.: The synthesis of α-amino-β-hydroxy-*n*-butyric acid, β-hydroxyvaline, β-hydroxynorvaline, etc. Ber. **67**, 530–47 (1934).

(3) ABDERHALDEN, E., KLARMANN, E., AND KOMM, E.: Further studies on the structure of the protein molecule. Z. physiol. Chem. **140**, 92–8 (1924).

(4) ADAMS, R., AND MARVEL, C. S.: Organic chemical reagents. VI. Reagents from butyl alcohol. J. Am. Chem. Soc. **42**, 310–20 (1920).

(5) ADAMSON, D. W.: New syntheses of basic amino acids and glycine. J. Chem. Soc. **1939**, 1564–8.

(6) ADKINS, H., AND REEVE, E. W.: A synthesis of *dl*-threonine. J. Am. Chem. Soc. **60**, 1328–31 (1938).

(7) ALBERTSON, N. F., AND ARCHER, S.: The use of ethylacetamidomalonate in the synthesis of amino acids. The preparation of *dl*-histidine, *dl*-phenylalanine, and *dl*-leucine. J. Am. Chem. Soc. **67**, 308–10 (1945).

(8) ALBERTSON, N. F., ARCHER, S., AND SUTER, C. M.: A new synthesis of tryptophane. J. Am. Chem. Soc. **66**, 500 (1944).

(9) ALBERTSON, N. F., ARCHER, S., AND SUTER, C. M.: The synthesis of tryptophane from gramine. J. Am. Chem. Soc. **67**, 36–7 (1945).

(10) ALBERTSON, N. F., AND TULLAR, B. F.: The synthesis of amino acids from acetamidocyanoacetic ester. J. Am. Chem. Soc. **67**, 502–3 (1945).

(11) ALDERSON, W. L.: Glycine. U. S. patent 2,346,547 (April 11, 1944).

(12) ANSLOW, W. K., AND KING, H.: Neutral salt addition compounds of alkaline earth glutamates and aspartates. Biochem. J. **21**, 1168–78 (1927).

(13) ANSLOW, W. K., KING, H., ORTEN, J. M., AND HILL, R. M.: Glycine. *Organic Syntheses*, Collective Volume 1, pp. 292–5. John Wiley and Sons, Inc., New York (1932).

(14) ANZIEGIN, A., AND GULEWIVICH, W.: The preparation of amino acids by electroreduction of oximino esters. Z. physiol. Chem. **158**, 32–41 (1926).

(15) AUBEL AND BOURGUEL: Alanine from pyruvic acid. Compt. rend. **186**, 1844–6 (1928).

(16) AUSTIN, P. R.: Liberation of amino acids such as glycine, valine, or β-alanine from their hydrohalides. U. S. patent 2,316,215 (April 13, 1943).

(17) BAEYER, A.: Experiments on uric acid group. Ann. **131**, 291–302 (1864).

(18) BAPTIST, N. G., AND ROBSON, W.: The salting-out of amino acids from protein hydrolysates. A method for the isolation of *l*-phenylalanine. Biochem. J. **34**, 221–8 (1940).

(19) BARGER, G., AND COYNE, F. P.: The amino acid methionine; constitution and synthesis. Biochem. J. **22**, 1417–25 (1928).

(20) Barger, G., and Weichselbaum, T. E.: A new synthesis of methionine. Biochem. J. **25**, 997–1000 (1931).

(21) Barker, A. L., and Skinner, G. S.: Deaminization of ester of alanine and of aminoisobutyric acid. J. Am. Chem. Soc. **46**, 403–14 (1924).

(22) Barnett, H. M.: Studies on leucine and dileucine hydrochloride and a new method for the isolation of leucine. J. Biol. Chem. **100**, 543–50 (1933).

(23) Barnett, H. M.: Leucine. U. S. patent 2,009,868 (July 30, 1935).

(24) Barnett, H. M.: Amino acids. U. S. patent 1,990,769 (February 12, 1935).

(25) Bartow, E., and Wetzstein, H. L.: Preparation of tyrosine from Steffen's waste. Proc. Iowa Acad. Sci. **47**, 193–5 (1940).

(26) Bauguess, L. C., and Berg, C. P.: Tryptophan metabolism. V. J. Biol. Chem. **104**, 675–89 (1934).

(27) Behrens, O. K.: Preparation of *l*-alanine from *dl*-alanine by the action of *d*-amino acid oxidase. J. Biol. Chem. **141**, 465–7 (1941).

(28) Behrens, O. K., Doherty, D. G., and Bergmann, M.: Resolution of *dl*-phenylalanine by asymmetric enzymic synthesis. J. Biol. Chem. **136**, 61–8 (1940).

(29) Benedict, H. C.: A note on the use of aniline in the preparation of amino acids. J. Am. Chem. Soc. **51**, 2277 (1929).

(30) Bergmann, M.: Complex salts of amino acids and peptides. II. Determination of *l*-proline with the aid of rhodanilic acid. The structure of gelatin. J. Biol. Chem. **110**, 471–9 (1935).

(31) Bergmann, M., Stern, F., and Witte, C.: Rearrangement of peptide-like substances. XIII. A new method of synthesis of dipeptides and dipeptide anhydrides. Ann. **449**, 277–302 (1926).

(32) Bergmann, M., and Zervas, L.: Aldehyde compounds of amino acids and their preparative application. Z. physiol. Chem. **152**, 282–99 (1926).

(33) Bergmann, M., and Zervas, L.: Synthesis of glycocyamine from arginine and glycine. Z. physiol. Chem. **172**, 277–88 (1927).

(34) Bergmann, M., and Zervas, L.: A general process for the synthesis of peptides. Ber. **65B**, 1192-1201 (1932).

(35) Bergmann, M., Zervas, L., and Salzmann, L.: Synthesis of *l*-asparagine and *d*-glutamine. Ber. **66B**, 1288-90 (1933).

(36) Billman, J. H., and Parker, E. E.: Amino acids. I. Glycine. J. Am. Chem. Soc. **65**, 761-2 (1943).

(37) Billman, J. H., and Parker, E. E.: Amino acids. II. Alanine. J. Am. Chem. Soc. **65**, 2455–6 (1943).

(38) Bishop, J. P., and Tucker, F. L.: Monosodium glutamate. U. S. patent 2,214,115 (September 10, 1940).

(39) Blicke, E.: "The Mannich Reaction" in *Organic Reactions* (edited by Roger Adams), Vol. 2. John Wiley and Sons, Inc., New York (1942).

(40) Block, P., Jr.: The conversion of diiodotyrosine to thyroxine. J. Biol. Chem. **135**, 51–2 (1940).

(41) Block, R. J.: A new method for the separation of the basic amino acids from protein hydrolysates. Proc. Soc. Exptl. Biol. Med. **51**, 252–3 (1942).

(42) Block, R. J.: Separation of amino acids. U. S. patent 2,386,926 (October 16, 1945).

(43) Block, R. J.: Amino acid separation. U. S. patent 2,387,824 (October 30, 1945).

(44) Block, R. J.: Separation of mineral acids from amino acids. U. S. patent application 446,840.

(45) Block, R. J.: Amino acid separation. U. S. patent application 650,121.

(46) Block, R. J.: Unpublished experiments.

(47) Block, R. J., and Bolling, D.: Food product. U. S. patent 2,344,229 (March 14, 1944).

(48) Block, R. J., and Bolling, D.: *The Amino Acid Composition of Proteins and Foods.* C. C. Thomas, Springfield, Illinois (1945).

(49) Block, R. J., and Hellwig, A. P.: Food product. U. S. patent 2,374,407 (April 25, 1945).

(50) Bolling, D.: Solubilization of proteins. U. S. patent application 571,371.

(51) Booth, E., Burnop, V. C. E., and Jones, W. E.: The synthesis of α-amino acids. I. *dl*-Methionine. J. Chem. Soc. **1944,** 666–7.

(52) Botvinnick, M. M., Morozova, E., and Samsonova, G.: Synthesis of β-hydroxynorvaline. Compt. rend. acad. sci. U. R. S. S. **30,** 133–6 (1941); Chem. Abstracts **35,** 4349 (1941).

(53) Boutwell, P. W., and Kuick, L. F.: A note on the preparation of glycine. J. Am. Chem. Soc. **52,** 4166–7 (1930).

(54) Bouveault, L., and Locquin, R.: A new procedure for the hydrogenation of oximes and the synthesis of a new leucine. Bull. soc. chim. [3] **35,** 965–9 (1906).

(55) Boyd, W. J., and Robson, W.: The synthesis of amino acids. I. Piperidine and diethylamine as catalysts in the condensation of aromatic aldehydes with hydantoins. Biochem. J. **29,** 542–5 (1935).

(56) Boyd, W. J., and Robson, W.: The synthesis of amino acids. II. Sulfides as reducing and hydrolytic agents in the hydantoin synthesis of amino acids. Biochem. J. **29,** 546–54 (1935).

(57) Boyd, W. J., and Robson, W.: The synthesis of indole-3-aldehyde and its homologues. Biochem. J. **29,** 555–61 (1935).

(58) Boyd, W. J., and Robson, W.: The synthesis of amino acids. III. Tryptophane. Biochem. J. **29,** 2256–8 (1935).

(59) Brand, E., and Sandberg, M.: *d*-Arginine hydrochloride. Org. Syntheses **12,** 4–9 (1932).

(60) Braun, J.v.: Synthesis of inactive lysine from piperidine. Ber. **42,** 839–46 (1909).

(61) Briggs, L. H., De Ath, G. C., and Ellis, S. R.: Reactions of hydrazoic acid. J. Chem. Soc. **1942,** 61–3.

(62) Brodkorb, F., Gluud, W., and Klempt, W.: Saponifying aminonitriles. German patent 655,563 (January 18, 1938).

(63) Bucherer, H. T., and Lieb, V. A.: Synthesis of hydantoins. II. Formation of substituted hydantoins from aldehydes and ketones. J. prakt. Chem. **141,** 5–43 (1934).

(64) Bucherer, H. T., and Steiner, W.: Synthesis of hydantoins. I. Reactions of α-hydroxy and α-amino nitriles. J. prakt. Chem. **140,** 291–316 (1934).

(65) Burch, W. J. N.: Hydroxyamino acids. J. Chem. Soc. **1930,** 310–12.

(66) Cahours, A.: Amino acids. Compt. rend. **44,** 567–71 (1855); **46,** 1044–7 (1858).

(67) Cannan, R. K.: The estimation of the dicarboxylic amino acids in protein hydrolysates. J. Biol. Chem. **152,** 401–10 (1944).

(68) Carter, H. E., and Hooper, I. R.: In M. Sahyun's *Amino Acids and Proteins.* Reinhold Publishing Corporation, New York (1944).

(69) Carter, H. E., and West, H. D.: *dl*-Threonine. Org. Syntheses **20,** 101–5 (1940).

(70) Chadwick, A. F., and Pacsu, E.: The rates of ammonolysis of some α-halogen acids and α-halogen acyl peptides. J. Am. Chem. Soc. **63,** 2427–31 (1941).

(71) Cheldelin, V. H., and Williams, R. J.: Adsorption of organic compounds. I. Adsorption of ampholytes on an activated charcoal. J. Am. Chem. Soc. **64,** 1513–16 (1942).

(72) Cheronis, N. D., and Spitzmueller, K. H.: Studies in ammonolysis. I. The ammonolysis of halogen fatty acids and the preparation of α-amino acids. J. Org. Chem. **6,** 349–75 (1941).

(73) Clarke, H. T., and Taylor, E. R.: α-Bromo-*n*-caproic acid. *Organic Syntheses,* Collective Volume 1, 108–9. John Wiley and Sons, Inc., New York (1932).

(74) Clarke, H. T.: In *Organic Chemistry* (edited by H. Gilman), Vol. II. John Wiley and Sons, Inc., New York (1938).

(75) Cocker, W.: Preparation of *dl*-asparagine and *dl*-aspartic acid. J. Chem. Soc. **1940,** 1489–91.

(76) Cocker, W., and Lapworth, A.: Experiments on the synthetic preparation and isolation of some of the simpler amino acids. J. Chem. Soc. **1931,** 1391–1403.

(77) Cocking, T. T.: Glycine—properties, manufacture and methods of identification. Ind. Chemist **13,** 137–8 (1937).

(78) Conrad, M., and Bischoff, C. A.: Syntheses with malonic ester. Ber. **13,** 595–607 (1880).

(79) Corn Products Refining Company: I. Glutamic acid. II. Amino acids. British patents 522,365 (June 17, 1940); 529,612 (November 25, 1940).

(80) Cox, G. J.: The preparation of *d*-arginine monohydrochloride. J. Biol. Chem. **78,** 475–9 (1928).

(81) Cox, G. J., and King, H.: *l*-Tryptophane. Org. Syntheses **10,** 100–5 (1930).

(82) Cox, G. J., King, H., and Berg, C. P.: The preparation of lysine, histidine, and arginine from hydrolyzed blood corpuscle paste by electrical transport. J. Biol. Chem. **81,** 755–64 (1929).

(83) Curtius, T.: Transformation of alkylated malonic acids into α-amino acids. J. prakt. Chem. [2] **125,** 211–302 (1930).

(84) Curtius, T., and Sieber, W.: Transformation of malonic acid into glycocoll and methylmalonic acid into β-alanine. Ber. **54B,** 1430–7 (1921).

(85) Curtius, T., and Sieber, W.: Transformation of alkylated malonic acids into α-amino acids. II. Synthesis of β-phenyl-α-alanine and of α-aminobutyric acid. Ber. **55B,** 1543–58 (1922).

(86) Dakin, H. D.: On amino acids. Biochem. J. **12,** 290–317 (1918).

(87) Dakin, H. D.: Hydroxyleucines. J. Biol. Chem. **154,** 549–55 (1944).

(88) Darapsky, A., Decker, H., Steuernagel, E., and Schiedrum, O: Preparation of α-amino acids from alkyl cyanoacetic esters. J. prakt. Chem. [2] **146,** 250–67 (1936).

(89) Darby, W. J., Lewis, H. B., and Totter, J. R.: The preparation of 4(5)-hydroxymethyl-imidazole. J. Am. Chem. Soc. **64,** 463–4 (1942).

(90) Desnuelle, P., and Fromageot, C.: New synthesis of glycine from glycolic acid. Bull. soc. chim. **1,** 700–2 (1934).

(91) Deulofeu, V.: Amino acids. V. A modification of the reduction of benzoylaminoacrylic acids in the Erlenmeyer synthesis. Anales soc. españ. fís. quím. **32,** 152–8 (1934).

(92) Doherty, D. G., Stein, W. H., and Bergmann, M.: Aromatic sulfonic acids as reagents for amino acids. J. Biol. Chem. **135,** 487–96 (1940).

(93) Drushel, W. A., and Knapp, D. R.: Preparation of glycocoll and diethyl carbonate. Am. J. Sci. **40,** 509–10 (1915).

(94) Dunn, M. S.: Chemistry of amino acids and proteins. Ann. Rev. Biochem. **10,** 91–124 (1941).

(95) Dunn, M. S.: In *The Chemistry of the Amino Acids and Proteins* (edited by C. L. A. Schmidt). C. C. Thomas, Springfield, Illinois (1943).

(96) Dunn, M. S., and Fox, S. W.: The synthesis of aspartic acid. J. Biol. Chem. **101,** 493–7 (1933).

(97) Dunn, M. S., Redemann, C. E., and Smith, N. L.: The synthesis of serine. J. Biol. Chem. **104,** 511–17 (1934).

(98) Dunn, M. S., and Smart, B. W.: A new synthesis of aspartic acid. J. Biol. Chem. **89,** 41–50 (1930).

(99) Dunn, M. S., Smart, B. W., Redemann, C. E., and Brown, K. E.: A new synthesis of glutamic acid. J. Biol. Chem. **94,** 599–609 (1931).

(100) Duschinsky, R.: A case of spontaneous resolution of a racemic compound (histidine monohydrochloride). Chemistry & Industry **12,** 10 (1934).

(101) Eck, J. C., and Marvel, C. S.: *dl*-Lysine hydrochlorides. Org. Syntheses **19,** 18–19, 20–3, 61–3 (1939).

(102) Ehrlich, F.: The naturally occurring isomer of leucine. Ber. **37,** 1809–40 (1904).

(103) Ehrlich, F.: The naturally occurring isomer of leucine. II. Ber. **40,** 2538–62 (1907).

(104) Elks, J., Elliott, D. F., and Hems, B. A.: Indole series. III. Improved synthesis of tryptophan. J. Chem. Soc. **1944**, 629–32.

(105) Ellinger, A., and Flamand, C.: The synthesis of tryptophan and some of its derivatives. Z. physiol. Chem. **55**, 8–24 (1908).

(106) Emerson, O. H., Kirk, P. L., and Schmidt, C. L. A.: The apparent dissociation constants of methionine and of isoserine. J. Biol. Chem. **92**, 449–52 (1931).

(107) Emmerling, A.: A new synthesis of glycine. Ber. **6**, 1351–4 (1873).

(108) Engel, M.: The conversion of maleic and fumaric acids into aspartic acid by direct fixation of ammonia. Bull. soc. chim. [2] **48**, 97–9 (1887).

(109) Engels, W. H., and Stein, G. A.: Amino carboxylic acids. U. S. patent 2,163,594 (June 27, 1939).

(110) Enkvist, T., and Laasonen, L.: Mercury salts as catalysts in the synthesis of aspartic acid from fumaric acid and ammonia. Ber. **72B**, 1927–32 (1939).

(111) Erlenmeyer, E., Jr.: The condensation of hippuric acid with phthalic acid anhydride and with benzaldehyde. Ann. **275**, 1–20 (1893).

(112) Erlenmeyer, E., Jr., and Halsey, J. T.: Two new syntheses of tyrosine. Ann. **307**, 138–46 (1899).

(113) Erlenmeyer, E., Jr., and Kunlin, J.: The formation of phenylacetylphenylalanine from the action of ammonia on phenylpyruvic acid. Ann. **307**, 146–51 (1899).

(114) Erlenmeyer, E., Jr., and Lipp, A.: Synthesis of tyrosine. Ann. **219**, 161–78, 179–233 (1883).

(115) Erlenmeyer, E., Jr., and Stoop, F.: The synthesis of serine and cystine. Ann. **337**, 236–63 (1904).

(116) Feofilaktov, V. V.: Synthesis of α-amino acids by means of alkylacetoacetic esters. Compt. rend. acad. sci. U.R.S.S. **24**, 755–8 (1939); Chem. Abstracts **34**, 1971 (1940).

(117) Feofilaktov, V. V.: Reaction between aromatic diazo compounds and compounds of the type of alkylacetoacetic esters. Bull. acad. sci. U.R.S.S. **1941**, 521-30; Chem. Abstracts **37**, 2347 (1943).

(118) Feofilaktov, V. V., and Blanko, F.: The action of diazobenzene upon esters of the acetoacetic type as a method for preparation of phenylhydrazones of α-keto acids and α-amino acids. VI. Synthesis of norleucine. J. Gen. Chem. (U.S.S.R.) **11**, 859–60 (1941); Chem. Abstracts **36**, 4096 (1942).

(119) Feofilaktov, V. V., and Onishchenko, A. S.: Synthesis of hydroxyproline (γ-hydroxypyrrolidine-α-carboxylic acid). Compt. rend. acad. sci. U.R.S.S. **20**, 133–5 (1938); Chem. Abstracts **33**, 1725 (1939).

(120) Feofilaktov, V. V., and Onishchenko, A. S.: The action of nitrous acid on α-substituted butyrolactones. I. J. Gen. Chem. (U.S.S.R.) **9**, 304–13 (1939); Chem. Abstracts **34**, 378 (1940).

(121) Feofilaktov, V. V., and Vinogradova, E.: Synthesis of phenylalanine from benzylmalonic and benzylcyanoacetic esters through the phenylhydrazone of phenylpyruvic acid. II. Compt. rend. acad. sci. U.R.S.S. **24**, 759–60 (1939); Chem. Abstracts **34**, 1971 (1940).

(122) Feofilaktov, V. V., and Zaĭtseva, V. N.: The action of benzenediazonium salts on alkylacetoacetic esters as a method for the preparation of phenylhydrazones of α-keto acids and α-amino acids. V. The synthesis of valine (α-aminoisovaleric acid). J. Gen. Chem. (U.S.S.R.) **10**, 1391–2 (1940); Chem. Abstracts **35**, 3606 (1941).

(123) Feofilaktov, V. V., and Zaĭtseva, V. N.: Action of aromatic diazo compounds on alkyl acetoacetic esters as a method for the synthesis of arylhydrazones of α-keto and of α-amino acids. VII. Synthesis of valine. VIII. Synthesis of tyrosine. J. Gen. Chem. (U.S.S.R.) **13**, 358–62 (1943); Chem. Abstracts **38**, 1211 (1944).

(124) Ferrari, C.: Compounds of glycine and alanine with picrates of the alkaline earths. Boll. sci. facoltà chim. ind. Bologna **1941**, 16–17.

(125) Fischer, E.: Synthesis of α,δ-diaminovaleric acid. Ber. **34**, 454–64 (1901).

(126) Fischer, E., and Boehner, R.: Conversion of glutamic acid or of pyrrolidonecarboxylic acid into proline. Ber. **44**, 1332–7 (1911).

(127) Fischer, E., and Groh, R.: Preparation of amino acids from keto acid phenylhydrazones and aluminum amalgam and the production of optically active γ-aminovaleric acid. Ann. **383**, 363–72 (1911).

(128) Fischer, E., and Raske, K.: Conversion of *l*-serine into natural cystine. Ber. **41**, 893–7 (1908).

(129) Fischer, E., and Weigert, F.: Synthesis of α, ε-diaminocaproic acid (inactive lysine) Ber. **35**, 3772–8 (1902).

(130) Fischer, E., and Zemplén, C.: Synthesis of both optically active prolines. Ber. **42**, 2989–97 (1909).

(131) Folin, O.: On the preparation of cystine. J. Biol. Chem. **8**, 9–10 (1910).

(132) Foreman, F. W.: The proline fraction in the hydrolysis of casein. Biochem. Z. **56**, 1–10 (1913).

(133) Foster, G. L., and Schmidt, C. L. A.: The separation of the dicarboxylic amino acids from certain hydrolysates by electrical transport. J. Am. Chem. Soc. **48**, 1709–14 (1926).

(134) Foster, G. L., and Shemin, D.: *l*-Histidine monohydrochloride. Org. Syntheses **18**, 43–6 (1938).

(135) Fox, S. W.: The preparation of citrulline by hydrolysis of arginine. J. Biol. Chem. **123**, 687–90 (1938).

(136) Frankel, N., and Kuk, S.: Remarks on the preparation of prolylalanine and prolylpeptides. Biochem. Z. **226**, 221–32 (1930).

(137) Freudenberg, K., and Huber, O.: Transformation of *d*-lactic acid into *l*-alanine. Ber. **58**, 148–50 (1925).

(138) Gabriel, S., and Kroseberg, K.: A simple synthesis of glycine. Ber. **22**, 426–8 (1889).

(139) Gagnon, P. E., Gaudry, R., and King, F. E.: Synthesis of amino acids from substituted cyanoacetic esters. J. Chem. Soc. **1944**, 13–15.

(140) Gaudry, R.: Synthesis of phenylalanine. Laval Med. **9**, 412 (1944).

(140A) Gaudry, R.: A synthesis of phenylalanine and tyrosine. Can. J. Research **23B**, 88–90 (1945).

(141) Gesellschaft für Kohlentechnik: Glycocoll. German patent 609,552 (February 21, 1935).

(142) Gilson, L. E.: A simplified method for preparing histidine. J. Biol. Chem. **124**, 281–5 (1938).

(143) Gluud, W., Löpmann, B., and Klempt, W.: Amino acids. German patent 653,009 (November 13, 1937).

(144) Gluud, W., Nüssler, W., and Brodkorb, F.: Glycine. German patent 572,803 (March 28, 1933).

(145) Goedeckemeyer, C.: Action of potassium phthalimide on some oxygen-containing halogen compounds. Ber. **21**, 2684–92 (1888).

(146) Gränacher, C.: The application of rhodanine to organic synthesis. I. Fusylalanine. Helv. Chim. Acta **5**, 610–24 (1922).

(147) Gränacher, C., Gero, M., Ofner, A., Klopfenstein, A., and Schlatter, E.: Use of rhodanine in organic syntheses. II. Amino acids and keto acids. Helv. Chim. Acta **6**, 458–67 (1923).

(148) Gurin, S.: High vacuum distillation of *N*-acyl amino acid and polypeptide esters. J. Am. Chem. Soc. **58**, 2104–6 (1936).

(149) Gurin, S., and Segal, C. F.: Helianthates of amino acid and polypeptide esters. J. Am. Chem. Soc. **58**, 2107–9 (1936).

(150) Gutknecht, H.: α-Nitrosopropionic acid. Ber. **13**, 1116–19 (1880).

(151) Hamlin, K. E., Jr., and Hartung, W. H.: The synthesis of α-amino acids from substituted acetoacetic esters. J. Biol. Chem. **145**, 349–57 (1942).

(152) Hanke, M. T., and Koessler, K. K.: Studies on proteinogenous amines. VI. The preparation of histidine from blood corpuscles. J. Biol. Chem. **43,** 521–6 (1920).

(153) Harington, C. R.: Resolution of *dl*-thyroxine. Biochem. J. **22,** 1429–35 (1928).

(153a) Harington, C. R.: Newer knowledge of the biochemistry of the thyroid gland. J. Chem. Soc. **1944,** 193–201.

(154) Harington, C. R., and Barger, G.: Thyroxine. III. Constitution and synthesis of thyroxine. Biochem. J. **21,** 169–83 (1927).

(155) Harington, C. R., and McCartney, W.: Note on the Erlenmeyer amino acid synthesis. Biochem. J. **21,** 852–6 (1927).

(156) Hass, H. B., and Marshall, J. R.: Synthesis from natural gas hydrocarbons. I. Caproic acid from pentane. Ind. Eng. Chem. **23,** 352–3 (1931).

(157) Heidelberger, M.: *dl*-α-Bromopropionic acid. *An Advanced Laboratory Manual of Organic Chemistry,* pp. 20–21. The Chemical Catalog Company, Inc., New York (1923).

(158) Henry, M. L.: On the composition of chlorobutyric acid. Bull. soc. chim. [2] **45,** 341–2 (1886).

(159) Herbst, R. M., and Shemin, D.: *dl*-β-Phenylalanine. α-Acetaminocinnamic acid. Org. Syntheses **19,** 1–3, 67–79 (1939).

(160) Heymons, A.: A simple method for the preparation of *dl*-proline. Ber. **66B,** 846–8 (1933).

(161) Hill, E. M., and Robson, W.: The salting out of amino acids from protein hydrolysates. I. The isolation of tyrosine, leucine, and methionine. Biochem J. **28,** 1008–15 (1934).

(162) Hill, E. M., and Robson, W.: A new synthesis of methionine and a scheme relating certain α-amino acids. Biochem. J. **30,** 248–51 (1936).

(163) Hoffmann-La Roche and Company: β-Aryl-α-aminopropionic acid. German patent 484,838 (September 7, 1928).

(164) Howe, E. E., Zambito, A. J., Snyder, H. R., and Tishler, M.: The application of a new alkylation reaction to the synthesis of tryptophane. J. Am. Chem. Soc. **67,** 38–9 (1945).

(165) Hüfner, G.: The identity of natural with synthetic leucine. J. prakt. Chem. **1,** 6–14 (1870).

(166) Hunter, A.: Enzymatic methods for the preparation of arginine and ornithine. Biochem. J. **33,** 27–35 (1939).

(167) I. G. Farbenindustrie A.-G.: Aliphatic amino acids. French patent 755,144 (November 20, 1933).

(168) Ikeda, K., and Suzuki, S.: U. S. patent 1,015,891 (January 30, 1912).

(169) Jay, R., and Curtius, R.: Methyleneaminoacetonitrile. Ber. **27,** 59–62 (1894).

(170) Johnson, T. B., and O'Brien, W. B.: Hydantoins. XIII. A new method for the synthesis of phenylalanine. J. Biol. Chem. **12,** 205–13 (1912).

(171) Johnson, T. B., and Tewkesbury, L. B.: Oxidation of 3,5-diiodotyrosine to thyroxine. Proc. Natl. Acad. Sci. U.S. **28,** 73–7 (1942).

(172) Jones, H. M.: A detailed method for the preparation of histidine. J. Biol. Chem. **33,** 429–31 (1918).

(173) de Jong, A. W. K.: Action of pyruvic acid on its ammonium salt. Rec. trav. chim. **19,** 259–310 (1900).

(174) Kapfhammer, J., and Spörer, H.: A new preparation of *l*-histidine from protein. Z. physiol. Chem. **173,** 245–9 (1928).

(175) Kavanagh, K. E.: Catalytic hydrogenation of cystine. J. Am. Chem. Soc. **64,** 2721 (1942).

(176) Keimatsu, S., and Sugasawa, S.: The synthesis of glutamic acid. I. J. Pharm. Soc. Japan **531,** 369–72 (1925).

(177) Kekulé, A.: The bromination products of succinic acid and their conversion into tartaric acid and malic acid. Ann. **117,** 120–9 (1861).

(178) Kendall, E. C., and McKensie, B. F.: *dl*-Alanine. Org. Syntheses **9**, 4–7 (1929).

(179) Kibrick, A. C.: The estimation of the dicarboxylic amino acids by titration. J. Biol. Chem. **152**, 411–18 (1944).

(180) King, F. E., and Robinson, R.: Experiments on the synthesis of physostigmine (eserine). VI. A synthesis of *dl*-eserinethole methopicrate. J. Chem. Soc. **1932**, 1433–8.

(181) King, H.: *d*-Glutamic acid. *Organic Syntheses*, Collective Volume 1, pp. 281–3. John Wiley and Sons, Inc., New York (1932).

(182) King, H.: 2-Acetyl-1-methylpyrrolidine. J. Chem. Soc. **1941**, 337–9.

(183) Kinney, C. R., and Adams, R.: Dideuteriovaline and dideuterioleucine. J. Am. Chem. Soc. **59**, 897–8 (1937).

(184) Klabunde, H. K.: Note on the preparation of hydroxyproline. J. Biol. Chem. **90**, 293–5 (1931).

(185) Knoop, F., and Landmann, G.: Synthesis of pseudoleucine. Z. physiol. Chem. **89**, 157–9 (1914).

(186) Knoop, F., and Oesterlin, H.: The natural synthesis of amino acids and its experimental reproduction. Z. physiol. Chem. **148**, 294–315 (1925).

(187) Knoop, F., and Oesterlin, H.: Synthesis and degradation of amino acids. Z. physiol. Chem. **170**, 186–211 (1927).

(188) Koessler, K. K., and Hanke, M. T.: Studies on proteinogenous amines. I. The synthesis of β-imidazolethylamine (histamine). J. Am. Chem. Soc. **40**, 1716–26 (1918).

(189) Kossel, A.: The constitution of the simplest proteins. Z. physiol. Chem. **25**, 165–89 (1898).

(190) Kossel, A., and Gross, R. E.: Preparation and estimation of arginine (in proteins). Sitzber. heidelberg. Akad. Wiss. **1B**, 1–6 (1923).

(191) Kossel, A., and Gross, R. E.: The preparation and quantitative determination of arginine. Z. physiol. Chem. **135**, 167–74 (1924).

(192) Kossel, A., and Kutscher, F.: Proteins. Z. physiol. Chem. **31**, 165–214 (1900–01).

(193) Krause, H.: Preparation of glycocoll. Chem.-Ztg. **55**, 666 (1931).

(194) Kuhn, H., and Stein, O.: Condensation of indoles with aldehydes and secondary amines. 1. A new gramine synthesis. Ber. **70A**, 567–9 (1937).

(195) Kuhn, R., and Quadbeck, G.: Synthetic preparation of lanthionine. Ber. **76B**, 527–8 (1943).

(196) Kurtz, A. C.: A simple synthesis of *dl*-citrulline. J. Biol. Chem. **122**, 477–84 (1938).

(197) Kurtz, A. C.: A new method for isolating *l*(+)-lysine. J. Biol. Chem. **140**, 705–10 (1941).

(198) Lamb, J., and Robson, W.: The Erlenmeyer synthesis of amino acids. Biochem. J. **25**, 1231–6 (1931).

(199) Lange, O.: A new compound formed from hydrocyanic acid. Ber. **6**, 99–101 (1873).

(200) Leuchs, H., and Bormann, K.: Pyrrolidine derivatives. V. Preparation of the three forms of hydroxyproline stereoisomeric with the natural form. Ber. **52B**, 2086–97 (1919).

(201) Leuchs, H., and Brewster, J. F.: Pyrrolidine derivatives. IV. Synthesis of natural hydroxyproline obtained from protein. Ber. **46**, 986–1000 (1913).

(202) Leuchs, H., Giua, M., and Brewster, J. F.: C_5 series. II. New cases of changes in configuration of the nature of the Walden inversion, etc. Ber. **45**, 1960–9 (1912).

(203) Levene, P. A., and Schormüller, A.: Synthesis of the phosphoric esters of hydroxyamino acids. III. J. Biol. Chem. **106**, 595–602 (1934).

(204) Levy, M., and Palmer, A. H.: The benzoylation and resolution of alanine. J. Biol. Chem. **146**, 493–5 (1942).

(205) Lewis, H. B., Chiles, H. M., and Cox, G. J.: *d*-Glutamic acid. Org. Syntheses **5**, 63–76 (1925).

(206) Li, L., Lin, K-H., Huang, Y-T., and Kang, S-A.: Chemical studies on amino acids and their derivatives. V. J. Chinese Chem. Soc. **9**, 1–40 (1942).

(207) Lichtenstein, N.: Preparation of *dl*-glutamine. Enzymologia **9**, 185–6 (1941).

(208) Ling, A. R., and Nanji, D. R.: Synthesis of glycine from formaldehyde. Biochem. J. **16**, 702–3 (1922).

(209) Lipp, A.: Some derivatives of isobutyraldehyde. Ann. **205**, 1–32 (1880).

(209a) Livak, J. E., Britton, E. C., Van der Weele, J. C., and Murray, M. F.: Synthesis of *dl*-methionine. J. Am. Chem. Soc. **67**, 2218–20 (1945).

(210) Lubavin, N. J.: The action of ammonium cyanide on aldehydes. J. Russ. Phys. Chem. Soc. **1**, 504 (1881); Ber. **14**, 2686–7 (1881).

(211) Locquin, R., and Cerchez, V.: Ethyl aminomalonate. Compt. rend. **186**, 1360–2 (1928).

(212) Locquin, R., and Cerchez, V.: Aminomalonic ester. Action of alkyl iodides and bromides on diethyl sodioaminomalonate. Bull. soc. chim. **47**, 1377–80, 1381–5 (1930).

(213) Locquin, R., and Cerchez, V.: Preparation of α-amino acids by hydrolysis of alkylaminomalonic esters. Bull. soc. chim. **47**, 1386–9 (1930).

(214) Lucas, C. C., and Beveridge, J. M. R.: The analysis of hair keratin. 1. A method for the quantitative removal of cystine from keratin hydrolysates. Biochem. J. **34**, 1356–66 (1940).

(215) Maeda, S., and Bunzi, M.: Synthesis and separation of amino acids. IV. Separation of hydroxyproline. Bull. Inst. Phys. Chem. Research (Tokyo) **17**, 271–3 (1938).

(216) Maeda, S., Terumi, M., and Suzuki, T.: Synthesis and separation of amino acids. III. Synthesis of serine. Bull. Inst. Phys. Chem. Research (Tokyo) **17**, 267–70 (1938).

(217) Majima, R., and Kotake, M.: Synthesis in the indole group. I. A new synthesis of racemic tryptophan. Ber. **55**, 2859–65 (1922).

(218) Majima, R., Unno, T., and Ono, K.: The reaction between acetylene and aniline at higher temperatures. Ber. **55**, 3854–9 (1922).

(219) Marshall, E. K., Jr.: The preparation of tyrosine. J. Biol. Chem. **15**, 85–6 (1913).

(220) Marvel, C. S.: *dl*-Valine. Org. Syntheses **20**, 106–8 (1940).

(221) Marvel, C. S.: *dl*-Isoleucine. Org. Syntheses **21**, 60–4 (1941).

(222) Marvel, C. S.: *dl*-Leucine (α-aminoisocaproic acid). Org. Syntheses **21**, 74–6 (1941).

(223) Marvel, C. S.: *dl*-Phenylalanine. Org. Syntheses **21**, 99–102 (1941).

(224) Marvel, C. S., and Stoddard, M. P.: A convenient synthesis of *dl*-glutamic acid. J. Org. Chem. **3**, 198–203 (1938).

(225) Marvel, C. S., and du Vigneaud, V.: α-Amino-*n*-caproic acid. Org. Syntheses **4**, 3–4 (1925).

(226) Marvel, C. S., and du Vigneaud, V.: α-Bromoisovaleric acid. Org. Syntheses **11**, 20–2 (1931).

(227) Masuda, R., Royal, C. L., and Marshall, A. E.: Process for manufacturing and recovering glutamic acid and its compounds. U. S. patent 1,967,563 (February 20, 1934).

(228) McIlwain, H., and Richardson, G. M.: Preparation of α-amino acids through the α-oximino esters. Biochem. J. **33**, 44–6 (1939).

(229) Menge, G. A.: Note on a new technique for the preparation of amino nitriles. J. Am. Chem. Soc. **56**, 2197–8 (1934).

(230) Minunni, G., and D'Urso, S.: New syntheses in the group of amino acids. I. Condensation of aldoximes with esters of β-ketonic acids. Gazz. chim. ital. **58**, 485–504 (1928).

(231) Mitra, S. K.: A new synthesis of serine. J. Indian Chem. Soc. **7**, 799–802 (1930).

(232) Müller, E.: Nitranilic acid as a precipitant for bases. Z. physiol. Chem. **268**, 245–50 (1941).

(233) Müller, E.: Base precipitation with 2-nitro-1,3-indandione. Z. physiol. Chem. **269,** 31–2 (1941).

(234) v. Mutzenbecher, P.: The formation of thyroxine from diiodotyrosine. Z. physiol. Chem. **261,** 253–6 (1939).

(235) Nierenburg, H.: A second synthesis of *d*-glutamine. Ber. **68B,** 2232–4 (1935).

(236) Noyes, W. A.: *n*-Butyl nitrite. *Organic Syntheses,* Collective Volume 2, pp. 108–9. John Wiley and Sons, Inc., New York (1943).

(237) Okabe, L.: Studies on the solubility of cystine under various conditions and a new method of cystine preparation. J. Biochem. (Japan) **8,** 441–57 (1928).

(238) Okuda, Y., and Kobayashi, T.: Determination and preparation of cystine. Bull. Agr. Chem. Soc. Japan **5,** 65–7 (1929).

(239) Onishchenko, A. S.: Action of nitrosyl chloride on alkylmalonic acids as a means of obtaining oximes of α-keto acids and of α-amino acids. I. Synthesis of phenylalanine and leucine. J. Gen. Chem. (U.S.S.R.) **11,** 197–202 (1941); Chem. Abstracts **35,** 7941 (1941).

(240) Orten, J. B., and Hill, R. M.: A simple method for the preparation of glycine. J. Am. Chem. Soc. **53,** 2797–9 (1931).

(241) Osterberg, A. E.: Ethyl phthalimidomalonate. *Organic Syntheses,* Collective Volume 1, pp. 266–7. John Wiley and Sons, Inc., New York (1932).

(242) Pacsu, E., and Mullen, J. W., 2nd: An improved method for the resolution of synthetic alanine. J. Biol. Chem. **136,** 335–42 (1940).

(243) Painter, E. P.: The synthesis of amino acids from benzoylaminomalonic ester. J. Am. Chem. Soc. **62,** 232–3 (1940).

(244) Patterson, W. I., and du Vigneaud, V.: The synthesis of homocystine. J. Biol. Chem. **111,** 393–8 (1935).

(245) Perkin, W. H., and Duppa, B. F.: The action of bromine on acetic acid. Ann. **108,** 106–13 (1858).

(246) Pirie, N. W.: The preparation of methionine from caseinogen. Biochem. J. **26,** 1270–4 (1932).

(247) Piutti, A.: Synthesis of aspartic acid. Gazz. chim. ital. **17,** 519–23 (1887).

(248) Piutti, A.: Preparation of asparagine by the diffusion method. Rend. accad. sci. (Napoli) **30,** 188–91 (1924).

(249) Pratt, A. E.: The preparation of *d*-arginine carbonate. J. Biol. Chem. **67,** 351–6 (1926).

(250) von Przylecki, S. T. J., and Kasprzyk, K.: A new method for separating amino acids. Biochem. Z. **289,** 243–50 (1936–37).

(251) Putochin, N. J.: The synthesis of proline. Ber. **56B,** 2213–16 (1923).

(252) Putokhin, N. I.: The catalytic reduction of pyrrole and its derivatives. The preparation of α-pyrrolidinemethylamine and of proline. J. Russ. Phys. Chem. Soc. **62,** 2216–25 (1930); Chem. Abstracts **25,** 3995 (1931).

(253) Pyman, F. L.: The synthesis of histidine. J. Chem. Soc. **99,** 1386–1401 (1911).

(254) Pyman, F. L.: Derivatives of glyoxaline 4(or 5)-formaldehyde and glyoxaline 4(or 5)-carboxylic acid. A new synthesis of histidine. J. Chem. Soc. **109,** 186–202 (1916).

(255) Redemann, C. E., and Dunn, M. S.: A general method for the synthesis of α-amino acids with ethyl benzamidomalonate. J. Biol. Chem. **130,** 341–8 (1939).

(256) Redemann, C. E., and Icke, R. N.: A convenient synthesis of *dl*-serine. J. Org. Chem. **8,** 159–61 (1943).

(257) Rice, E. E.: A simplified procedure for the isolation of lysine from protein hydrolysates. J. Biol. Chem. **131,** 1–4 (1939).

(258) Robertson, S. R.: The reaction of chloracetic acid with ammonia and the preparation of glycine. J. Am. Chem. Soc. **49,** 2889–94 (1927).

(259) Ruggli, P., and Businger, A.: Synthesis of β-alanine. Helv. Chim. Acta **25,** 35–9 (1942).

(260) Sadikov, V. S., and Lindkvist-Ruisakova, E. V.: Behavior of diamino acids from casein hydrolysates toward permutit. Compt. rend. acad. sci. U. R. S. S. **1**, 575–7 (1934); Chem. Abstracts **28**, 5842 (1934).

(261) Sah, P. P. T.: Synthesis of amino acids. I. Application of Curtius reaction to the synthesis of glycine. J. Chinese Chem. Soc. **4**, 198–207 (1936).

(262) Sakai, K., and Wada, S.: Tryptophan. Japanese patent 37,870 (January 22, 1921).

(263) Sannie, C.: The mechanism of the synthesis of the α-amino acids by Strecker's reaction. II. The disappearance of ammonia and the intermediate reactions. Bull. soc. chim. **39**, 254–78 (1926).

(264) Sasaki, R.: Condensation of glycine anhydride with aldehydes. A new synthesis of *dl*-phenylalanine and *dl*-tyrosine. Ber. **54**, 163–8 (1921).

(265) Savitzkii, A. Y.: Thyroxine from hydroquinone monomethyl ether and 3,4,5-triiodonitrobenzene. Med. exptl. (Ukraine) **1**, 39–49 (1934); Chem. Abstracts **30**, 1761 (1936).

(266) Savitskii, A. Y.: The preparation and properties of 3,5-diiodo-*l*-tyrosine. J. Gen. Chem. (U.S.S.R.) **9**, 1342–4 (1939); Chem. Abstracts **34**, 742 (1940).

(267) Schaaf, K. H., and Pickel, F. D.: Synthesis of amino acids. U. S. patent 2,365,295 (December 19, 1944).

(268) Schiltz, L. R., and Carter, H. E.: Synthesis of serine. J. Biol. Chem. **116**, 793–7 (1936).

(269) Schmidt, C. L. A.: "Synthesis of isotopic proline", in *The Chemistry of the Amino Acids and Proteins*. C. C. Thomas, Springfield, Illinois (1943).

(270) Schmidt, C. L. A.: A method for the preparation of cystine. Proc. Soc. Exptl. Biol. Med. **19**, 50–2 (1921).

(271) Schmidt, J., and Widmann, K. T.: True aliphatic nitrosocarbonic esters. Ber. **42B**, 1886–1902 (1909).

(272) Schmidt, K. F.: The imine residue. Ber. **57**, 704–6 (1924).

(273) Schniepp, L. E., and Marvel, C. S.: Some reactions of γ-aminovaleric acid and its derivatives. J. Am. Chem. Soc. **57**, 1557–8 (1935).

(274) Schoenheimer, R., and Ratner, S.: Studies in protein metabolism. III. Synthesis of amino acids containing isotopic nitrogen. J. Biol. Chem. **127**, 301–13 (1939).

(275) Schrauth, W., and Geller, H.: Preparation of β-hydroxy-α-amino acids from olefincarboxylic acids. Ber. **55**, 2783–96 (1922).

(276) Schroeter, G.: Simple synthesis of α-aminocarboxylic acids. Z. angew. Chem. **39**, 1460 (1926).

(277) Schroeter, G.: Amino acids. German patent 491,292 (September 18, 1926).

(278) Schryver, S. B., Buston, H. W., and Mukherjee, D. H.: The isolation of a product of hydrolysis of the proteins hitherto undescribed. Proc. Roy. Soc. (London) **B98**, 58–65 (1925).

(279) Senter, G., Drew, H. D. K., and Marten, G. H.: Walden inversion. VII. Influence of the solvent on the sign of the product in the conversion of α-bromo-β-phenylpropionic acid and α-amino-β-phenylpropionic acid (phenylalanine). Iminodiphenyldipropionic acid. J. Chem. Soc. **113**, 151–63 (1918).

(280) Shemin, D., and Herbst, R. M.: The synthesis of dipeptides from α-keto acids. J. Am. Chem. Soc. **60**, 1951–4 (1938).

(281) Shildruck, P. R.: Glutamic acid. U. S. patent 2,347,220 (April 25, 1944).

(282) Signaigo, F. K., and Adkins, H.: A synthesis of *dl*-proline from pyrrole. J. Am. Chem. Soc. **58**, 1122–4 (1936).

(283) Sisler, H. H., and Cheronis, N. D.: Ammonolysis of α-halogen acids in liquid ammonia. (Studies in ammonolysis.) J. Org. Chem. **6**, 467–78 (1941).

(284) Skita, A., and Wulff, C.: Syntheses of amino acids. Ann. **453**, 190–210 (1927).

(285) Snyder, H. R., Andreen, J. H., Cannon, G. W., and Peters, C. F.: A convenient synthesis of *dl*-methionine. J. Am. Chem. Soc. **64**, 2082–4 (1942).

(286) Snyder, H. R., and Cannon, G. W.: A new synthesis of homocystine and a further improvement in the synthesis of methionine. J. Am. Chem. Soc. **66**, 511–12 (1944).

(287) Snyder, H. R., and Chiddix, M. E.: Synthetic amino acids. Some reactions of 3,6-bis(β-chloroethyl)-2,5-diketopiperazine. J. Am. Chem. Soc. **66**, 1000–2, 1002–4 (1944).

(288) Snyder, H. R., Howe, E. E., Cannon, G. W., and Nyman, M. A.: ω,ω'-Bimethionine. J. Am. Chem. Soc. **65**, 2211–14 (1943).

(289) Snyder, H. R., Shekleton, J. F., and Lewis, C. D.: Synthetic amino acids. Syntheses from acetamidomalonic ester. J. Am. Chem. Soc. **67**, 310–12 (1945).

(290) Snyder, H. R., and Smith, C. W.: A convenient synthesis of *dl*-tryptophan. J. Am. Chem. Soc. **66**, 350–1 (1944).

(291) Snyder, H. R., Smith, C. W., and Stewart, J. M.: Carbon alkylation with quaternary ammonium salts. A new approach to the synthesis of compounds containing the β-indolemethylene group. J. Am. Chem. Soc. **66**, 200–4 (1944).

(292) Sørensen, S. P.: The synthesis of amino acids. Compt. rend. trav. lab. Carlsberg **6**, 1–60 (1903).

(293) Sørensen, S. P. L.: Syntheses of amino acids by means of phthalimidomalonic ester. Z. physiol. Chem. **44**, 448–60 (1905).

(294) Sørensen, S. P. L.: Synthesis of *dl*-arginine (α-amino-δ-guanido-*n*-valeric acid) and the isomeric α-guanido-δ-aminovaleric acid. Ber. **43**, 643–51 (1910).

(295) Sørensen, S. P. L.: Syntheses of amino acids. XI. α-Amino-γ,δ-dihydroxyvaleric acid, γ-hydroxyproline, and γ,δ-diamino-γ-hydroxyvaleric acid (γ-hydroxyornithine. Compt. rend. trav. lab. Carlsberg **11**, 121–2 (1916).

(296) Sørensen, S. P. L., and Andersen, A. C.: Amino acid syntheses. VII. Proline (α-pyrrolidinecarbonic acid). Z. physiol. Chem. **56**, 236–49 (1908).

(297) Sørensen, D. P. L., Høyrup, M., and Andersen, A. C.: Synthesis of amino acids. IX. *dl*-Arginine (α-amino-δ-guanidinovaleric acid) and the isomeric δ-amino-α-guanidinovaleric acid. Z. physiol. Chem. **76**, 44–94 (1912).

(298) Stephen, H., and Weizmann, C.: Synthesis of *dl*-tyrosine and *dl*-3,4-dihydroxyphenylalanine. J. Chem. Soc. **105**, 1152–5 (1914).

(299) Stein, W. H., and Bergmann, M.: Determination of proline in mixtures containing *l*- and *dl*-proline. The proline content of gelatin. J. Biol. Chem. **134**, 627–33 (1940).

(300) Stein, W. H., Moore, S., and Bergmann, M.: The isolation of *l*-serine from silk fibroin. J. Biol. Chem. **139**, 481–2 (1941).

(301) Stein, W. H., Moore, S., Stamm, G., Chou, C., and Bergmann, M.: Aromatic sulfonic acids as reagents for amino acids. The preparation of *l*-serine, *l*-alanine, *l*-phenylalanine, and *l*-leucine from protein hydrolysates. J. Biol. Chem. **143**, 121–9 (1942).

(302) Strecker, A.: The synthesis of lactic acid and a new glycine homologue. Ann. **75**, 27–45 (1850).

(303) Strecker, H.: Some compounds of valeraldehyde. Ann. **130**, 217–23 (1864).

(304) Sugasawa, S.: Synthesis of amino acids. VI. Synthesis of *dl*-lysine. J. Pharm. Soc. Japan **550**, 1044–50 (1927).

(305) Tarver, H., and Schmidt, C. L. A.: Radioactive sulfur studies. I. Synthesis of methionine. J. Biol. Chem. **146**, 69–84 (1942).

(306) Taylor, R. S., and Connor, R.: The Michael condensation. VII. Activation of the methylene group by carbon–carbon unsaturation. J. Org. Chem. **6**, 696–704 (1941).

(307) Tiemann, F.: Aromatic amino acids. Ber. **13**, 381–5 (1880).

(308) Tiemann, F.: The preparation of amino acids from the cyanohydrins of aldehydes and ketones. Ber. **14**, 1957–85 (1881).

(309) Tobie, W. C., and Ayres, G. B.: Synthesis of *dl*-alanine in improved yield from α-bromopropionic acid and aqueous ammonia. J. Am. Chem. Soc. **59**, 950 (1937).

(310) Tobie, W. C., and Ayres, G. B.: An improved procedure for the preparation of glycine. J. Am. Chem. Soc. **64,** 725 (1942).

(311) Toennies, G.: Acid hydrolysates of proteins. British patent 527,320 (October 7, 1940).

(312) Town, B. W.: The isolation of pure *l*-proline. Biochem. J. **22,** 1083–6 (1928).

(313) Town, B. W.: The micro-determination of glycine in protein hydrolysates. Biochem. J. **30,** 1833–6 (1936).

(314) Town, B. W.: The 3,5-dinitrobenzoyl derivatives of the amino acids and their use in separating the isomers of leucine and valine. Biochem. J. **35,** 578–87 (1941).

(315) Traube, W., Johow, R., and Tepohl, W.: α,δ-Diamino-γ-valerolactone and a new synthesis of hydroxyproline. Ber. **56B,** 1861–6 (1923).

(316) Traube, W., and Lehmann, E.: Combination of alkylene oxides with malonic ester and acetoacetic ester. Ber. **34,** 1971–83 (1901).

(317) Turba, F.: The adsorption behavior of protein-split products. 1. Chromatography of the basic amino acids on bleaching earths. Ber. **74B,** 1829–38 (1941).

(318) Turba, F., and Richter, M.: The adsorption behavior of protein-split products. II. Chromatography of dicarboxylic amino acids on aluminum oxide. Ber. **75,** 340–4 (1942).

(319) Tutiya, Y.: Synthesis of aspartic acid. J. Agr. Chem. Soc. Japan **17,** 706–10 (1941).

(320) Van Slyke, D. D., Hiller, A., Dillon, R. T., and MacFadyen, D.: The unidentified base in gelatin. Proc. Soc. Exptl. Biol. Med. **38,** 548–9 (1938).

(321) Vickery, H. B.: The preparation of histidine by means of 3,4-dichlorobenzenesulfonic acid. J. Biol. Chem. **143,** 77–87 (1942).

(322) Vickery, H. B., and Leavenworth, C. S.: On the separation of histidine and arginine. IV. The preparation of histidine. J. Biol. Chem. **78,** 627–35 (1928).

(323) Vickery, H. B., Pucher, G. W., and Clark, H. E.: The preparation of glutamine. J. Biol. Chem. **109,** 39–42 (1935).

(324) Vickery, H. B., Pucher, G. W., and Deuber, C. G.: The preparation of asparagine. J. Biol. Chem. **145,** 45–53 (1942).

(325) du Vigneaud, V., McKennis, H., Simmonds, S., Dittmer, K., and Brown, G. B.: The inhibition of the growth of yeast by thienylalanine. J. Biol. Chem. **159,** 385–97 (1945).

(326) Walden, P.: Optical activity of chlorofumaric acid and optically active halogenated succinic acids. Ber. **26,** 210–15 (1893).

(327) Waterman, H. C.: The preparation of tryptophane from the products of hydrolysis of lactalbumin with baryta. J. Biol. Chem. **56,** 75–7 (1923).

(328) Weidenhagen, R., and Herrmann, R.: A new synthesis of imidazoles. Ber. **68,** 1953–61 (1935).

(329) Weidenhagen, R., Herrmann, R., and Wegner, H.: Imidazoles. Ber. **70,** 570–83 (1937).

(330) Weidinger, A.: Quantitative method for the isolation of *l*-cystine from keratin (horse hair). Rev. trav. chim. **56,** 562–4 (1937).

(331) West, H. D., and Carter, H. E.: Synthesis of α-amino-β-hydroxy-*n*-butyric acids. IV. Separation of mixtures of the two forms and preparation of *d*(−)- and *l*(+)-threonine. J. Biol. Chem. **119,** 109–19 (1937).

(332) Weygand, F.: Preparation of β-alanine. Ber. **74B,** 256–7 (1941).

(333) Wheeler, H. L., and Hoffman, C.: Hydantoins: Synthesis of phenylalanine and of tyrosine. Am. Chem. J. **45,** 368–83 (1911).

(334) Wheeler, H. L., and Jameson, G. S.: Synthesis of iodogorgoic acid. Am. Chem. J. **33,** 365–72 (1905).

(335) Wheeler, H. L., and Mendel, L. B.: The iodine complex in sponges (3,5-diiodotyrosine). J. Biol. Chem. **7,** 1–9 (1909).

(336) Willstätter, R.: Synthesis of hygric acid. Ber. **33,** 1160–6 (1900).

(337) WILLSTÄTTER, R., AND ETTLINGER, F.: Syntheses of hydric acid and α-pyrrolidincarbonic acid. Ann. **326,** 91–128 (1903).

(338) WINDUS, W., AND MARVEL, C. S.: A synthesis of methionine. J. Am. Chem. Soc. **52,** 2575–80 (1930).

(339) WOLFF, J.: Aspartic acid from maleic acid. Ann. **75,** 293–7 (1850).

(340) WOOD, J. L., AND DU VIGNEAUD, V.: A new synthesis of cystine. J. Biol. Chem. **131,** 267–71 (1939).

(341) WOOD, J. L., AND DU VIGNEAUD, V.: On the synthesis of serine. J. Biol. Chem. **134,** 413–16 (1940).

(342) YU-CHING, CHENG, AND ADOLPH, W. H.: Note on the preparation of *d*-glutamic acid. J. Chinese Chem. Soc. **2,** 221–4 (1934).

(343) ZEYNEK, R.: The preparation of chloro- and bromo-tyrosine and of the analogous tyramines. Z. physiol. Chem. **114,** 275–85 (1920).

I. A. R. I. 75.

IN[illegible]GRICULTURAL RESEARCH
INSTITUTE LIBRARY
NEW DELHI.